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ORIGINAL ARTICLE

Associations between Vitamin D and β-Cell Function and Colorectal Cancer-Associated Tumor Markers in Chinese Type 2 Diabetic Patients with Albuminuria

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SUMMARY

Background: **This study is to investigate the protective effects of vitamin D in T2DM, as well as the associations between serum calcifediol level and β-cell function, and risk of CRC, in Chinese type 2 diabetes mellitus (T2DM) patients with albuminuria.**
Methods: **Serum calcifediol levels were analyzed and compared among healthy individuals and T2DM patients stratified by albumin/creatinine ratio (ACR). Relative correlation analyses were performed with β-cell function (BCF) and risk of CRC.**
Results: **Patients' ACR was positively associated with fasting plasma glucose and insulin, homeostasis model assessment (HOMA) of insulin resistance (IR), alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA), carbohydrate antigen (CA)199, CA125, and Septin9 methylation, but inversely associated with HOMA-BCF and insulin secretion. Serum calcifediol level in the healthy controls was significantly higher than T2DM patients. In T2DM patients, calcifediol level was inversely associated with ACR, HOMA-IR, AFP, CEA, and Septin9 methylation, but positively associated with HOMA-BCF and insulin secretion. Multivariate stepwise principal component regression analysis indicated that calcifediol, hemoglobin A1c, and serum creatinine were independent risk factors for elevated CEA in T2DM.**
Conclusions: **Higher serum calcifediol level is correlated with better β-cell function, lower insulin resistance, and decreased risk of CRC. Vitamin D may have suppressive effects on T2DM-associated complications and therefore represents a potential prophylactic treatment against β-cell dysfunction and cancer development in T2DM patients with albuminuria.**
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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder with steadily increasing prevalence worldwide nowadays [1,2]. T2DM commonly occur among older population; however, in recent years, the disease has been more and more frequently identified in younger population [3]. Low sensitivity to insulin and pancreatic β-cell failure represent the two major causes for T2DM.

At present, there is still no cure for T2DM, and common treatments usually aim to lower blood sugar, increase insulin sensitivity, and boost insulin production. Chemical agents mainly used in the clinical treatment of T2DM mainly include alpha-glucosidase, dipeptidyl peptidase 4 (DPP-4) and sodium-glucose co-transporter 2 (SGLT2) inhibitors, and biguanides. However, these medications need to be taken throughout the lifetime, and their long-term side effects are still not clear. In addition, the cost of prescription medications, and the possible medication tolerance and the need to switch medications, would also cause great burdens to the diabetic patients, family, and society.

Vitamin D is an alternative medicine or natural remedy that has attracted increasing attention recently due to its regulatory roles on various biological functions and/or processes, including calcium and bone metabolism, renin-angiotensin system, insulin resistance, vasculature, and immune system [4]. Vitamin D can be naturally acquired from food intake (10 - 20%) such as milk products and meat, but most of it (80 - 90%) is obtained through the skin exposure to the sun. Ultraviolet radiation converts the vitamin D precursor in the skin into vitamin D3 (cholecalciferol), which is then hydroxylated into calcifediol (also known as 25-hydroxycholecalciferol, 25-hydroxyvitamin D3, or 25[OH]D) in the liver. Calcifediol is an inactive metabolite of vitamin D, which is also considered as a circulating indicator for vitamin D in the body. This metabolite could be converted into the active product calcitriol (also known as INN, 1,25-dihydroxyvitamin D3, or 1,25[OH]2D) by 1α-hydroxylase in the kidney and circulated via the blood to the target tissues and/or cells.

Vitamin D deficiency has been associated with increased risk of T2DM-associated complications. Moreover, in T2DM patients, along with the increasing calcifediol levels, albuminuria (indicated by the albumin/creatinine ratio, ACR) would be decreased [5-7]. Furthermore, low levels of calcifediol and albuminuria have been associated with higher risk of colorectal cancer (CRC) [8-11]. A meta-analysis of studies (combined study population of 17,664 subjects) revealed that patients with T2DM, especially those with diabetic nephropathy, often have decreased serum levels of VD [12] and decreased serum levels of VD were shown to increase the risk of malignant tumors. A recent double-blinded clinical trial from Momeni et al. [13] has suggested that the vitamin D therapy may decrease proteinuria in T2DM patients. This is supported by an earlier report showing that administration of vitamin D3 may have an antiproteinuric effect in Chinese diabetic patients [14]. Colorectal cancer (CRC) is a malignant tumor that predominantly occurs in middle aged and elderly people [15]. Patients typically exhibit no obvious clinical symptoms in the early stages of the disease. Screening of serum markers is an important tool for early diagnosis of CRC [16]. At present, the more commonly used serum markers include carbohydrate antigen AFP, CEA, (CA) 19-9, CA125, CA153, and CA724.

Septin9 is a gene that was shown to be directly related with occurrence of cancer. Methylation of the CpG islands in the 5' region of the Septin9 gene has been implicated in the development of CRC. The incidence of hypermethylation in CRC tissue is up to 90% compared to 10% in normal control tissues [17]. Therefore, the Septin9 methylation is an important biological characteristic and serological marker for CRC. Methylated Septin9 (mS9) DNA can be released from the necrotic or apoptotic tumor cells into the peripheral circulation in the early stages of CRC. Thus, detection of mS9 in peripheral blood can be used for screening, early diagnosis, treatment evaluation, and dynamic monitoring of CRC [18,19].

In this study, to gain insights into the protective effects of vitamin D in T2DM, the associations between calcifediol level and β-cell function, and risk of CRC, in Chinese T2DM patients with different levels of albuminuria, were investigated.

MATERIALS AND METHODS

Study subjects

The study population herein included patients with T2DM (n = 320) and age-matched healthy individuals (control; n = 150). These patients were diagnosed with T2DM and treated at the Department of Health and Endocrinology of Jinan Central Hospital Affiliated to Shandong University from January 2016 to November 2017. The control subjects underwent physical examinations at the Jinan Central Hospital Affiliated to Shandong University during the same time period.

Exclusion criteria were as follows: acute diabetic complications, such as diabetic hyperosmolar coma and diabetic ketoacidosis, within the previous 3 months; cardiovascular and cerebrovascular diseases, liver and kidney diseases, infectious diseases, malignant tumor, autoimmune diseases, or other systemic diseases; or recent stress conditions, including infections, surgery, trauma, pregnancy, and breast-feeding. Moreover, individuals who had taken calcium supplements, active vitamin D preparations, glucocorticoids, or estrogen within the previous 3 months; or drugs affecting kidney function within the previous 2 weeks, were also excluded from this study. Prior written and informed consent were obtained from every patient and the study was approved by the ethics review board of Jinan Central Hospital Affiliated to Shandong University.

Randomization and measurements

The included diabetic patients were divided into the normoalbuminuria (n = 113), microalbuminuria (n = 102), and macroalbuminuria (n = 105) groups based on ACR. Normoalbuminuria, microalbuminuria, and macroalbuminuria were defined as ACR < 30, between 30 and 300, and > 300 mg/g, respectively. General information was collected, including age, height, weight, and

blood pressure.

Fasting (12 hours) blood sample was collected from the basilic vein of each patient in the morning, which was subjected to the following measurements: fasting plasma glucose (FPG); fasting insulin (FINS); hemoglobin A1c (HbA1c); high-density lipoprotein cholesterol (HDL-C); low-density lipoprotein cholesterol (LDL-C); total cholesterol (TC); triglyceride (TG); serum uric acid (SUA); serum creatinine (SCr); blood urine nitrogen (BUN); alpha-fetoprotein (AFP); carcinoembryonic antigen (CEA); carbohydrate antigen (CA)199; CA125; CA153; and CA724 levels. Morning urine (5 mL) was collected to measure the urine albumin and creatinine levels.

Body mass index (BMI) and ACR were calculated. Insulin resistance (IR) was evaluated by the homeostasis model assessment (HOMA) using the following formulation: HOMA-IR = (FINS x FPG)/22.5. Insulin active index (IAI = ln [1/[FINS x FPG]]), β-cell function index (HOMA-BCF = 20 x FINS/FPG - 3.5), and insulin secretion index (ISI = FINS/FPG) were also obtained.

Enzyme-linked immunosorbent assay (ELISA)

The serum calcifediol level was measured by the ELISA kit (Beijing Rongzhi Haida Biotech, Beijing, China), according to the manufacturer's instructions. The fluorescence was detected with the Bio-Rad model 680 microplate reader (Bio-Rad Laboratories, Hercules, CA, USA).

Electrochemiluminescence

Serum 25-OH-D concentration was detected by electrochemiluminescence with the 25-OH-D detection kit (Roche). After 3 rounds of incubation, the 25-OH-D-containing complex was bound to the solid supporting materials under the interaction of biotin and streptavidin. For the reaction, the reaction solution was sucked into the measuring pool, and the magnetic beads were absorbed onto the surface of the electrodes. Substances not bound to the magnetic beads were removed by the ProCell/ProCell M solution. A certain voltage was applied to the electrodes to stimulate the chemiluminescence, and the luminescence intensity was detected with the Cobas e411 analyzer (Roche).

Quantitative real-time PCR

Methylation of the colon cancer *Septin9* gene (SEPT9; on chromosome 17) in blood samples was measured in triplicate using the probe-based real-time PCR (qPCR) with the Septin9 Methylation Detection Kit (Biochain Beijing Technology Co., Ltd., Beijing, China). For details, 10 mL peripheral blood sample was collected from fasting patients in the morning. DNA12 was extracted using the plasma treatment kit according to the manufacturer's instructions. The sulfite solution was complexed with the DNA eluate. The methylated DNA was directly extracted by the magnetic bead adsorption, and the non-methylated DNA was converted by the deamination reaction. The PCR was performed on the ABI 7,500 thermocycler (Applied Biosystems). The reaction conditions were as follows: 94°C for 20 minutes; 62°C for 5 seconds, 55.5°C for 35 seconds, 93°C for 30 second, for a total of 45 cycles; followed by 40°C for 5 seconds. The results were interpreted using the 7,500 Fast PCR software. Experiment was performed in triplicates.

Statistical analysis

Statistical analysis was performed using the SPSS17.0 software. All variables were tested for normal distribution. Normally distributed measured data were expressed as mean ± SD, which were compared using the one-way analysis of variance (between groups) and Fisher's least significant difference test (pairwise comparisons). Non-normally distributed measured data were expressed as median (interquartile range), which were statistically analyzed after logarithmic conversion. Correlations between serum calcifediol level and various T2DM parameters were determined using the Pearson's correlation, partial correlation, and multiple linear regression analyses. $p < 0.05$ was considered statistically significant.

RESULTS

General information and clinical characteristics

There were no significant differences in the age and gender ratio between the T2DM patients and healthy control subjects (Table 1). Compared with the healthy control group, the FPG, FINS, and HbA1c levels were significantly elevated in these T2DM groups. In particular, the FPG, FINS, and HbA1c levels in the microalbuminuria and macroalbuminuria groups were also significantly higher than the normoalbuminuria group. Moreover, the LDL-C, TC, SUA, SCr, and BUN levels were comparable between the normoalbuminuria and microalbuminuria groups. However, the levels of these indicators in the macroalbuminuria group were significantly higher than the normoalbuminuria, microalbuminuria, and healthy control groups. These results suggest that, the FINS, FPG, and HbA1c levels, in the T2DM patients, are significantly higher than healthy individuals, which are positively associated with ACR.

Serum calcifediol levels

Serum calcifediol levels were next investigated and analyzed. Our results showed that the serum calcifediol levels in healthy controls were significantly higher than T2DM patients (Table 1). Furthermore, serum calcifediol level in the normoalbuminuria group was significantly higher than the microalbuminuria group, which was also significantly higher than the macroalbuminuria group. The correlation analysis showed that the serum calcifediol level was significantly negatively correlated with the FPG and FINS levels (Table 5). These results suggest that in the T2DM patients with higher ACR lower serum calcifediol level is associated with poorer

Table 1. Clinical characteristics and biochemical data of study subjects.

	Control	Diabetic patients		
		Normal ACR	Microalbuminuria	Macroalbuminuria
Total, n	150	113	102	105
Men/women, n/n	76/74	48/65	55/47	47/58
Age, year	60.03 ± 12.07	63.92 ± 10.64	63.31 ± 14.01	60.57 ± 10.38
BMI, kg/m^2	25.01 ± 2.18	25.24 ± 2.12	26.04 ± 3.53 [a, b]	25.42 ± 2.77
FPG	4.86 ± 0.26	7.19 ± 2.09 [a]	9.29 ± 3.85 [a, b]	9.13 ± 2.37 [a, b]
HbA1c, %	5.53 ± 0.25	8.16 ± 1.34 [a]	9.22 ± 1.23 [a, b]	9.27 ± 0.42 [a, b]
HDL-C, mmol/L	1.33 ± 0.33	1.22 ± 0.31	1.06 ± 0.24 [a, b]	1.05 ± 0.23
LDL-C, mmol/L	2.70 ± 0.99	3.06 ± 1.00	3.12 ± 0.98	3.76 ± 0.73 [a, b, c]
TC, mmol/L	4.24 ± 1.08	4.70 ± 1.14	5.10 ± 1.13 [a, b]	5.69 ± 0.53 [a, b, c]
TG, mmol/L	1.23 ± 0.70	1.38 ± 0.77	2.13 ± 1.46 [b]	2.65 ± 0.79 [a, b]
SUA μmol/L	305.71 ± 89.91	307.73 ± 105.11	320.58 ± 99.13	410.72 ± 117.39 [a, b, c]
SCr, μmol/L	61.97 ± 13.86	62.95 ± 16.95	69.32 ± 22.89	98.62 ± 45.60 [a, b, c]
BUN, mmol/L	4.67 ± 1.30	4.80 ± 1.61	6.72 ± 6.82	9.50 ± 4.11 [a, b, c]
FINS, mIU/L	5.08 ± 3.49	12.02 ± 7.42 [a]	14.64 ± 8.17 [a, b]	15.63 ± 7.27 [a, b]
Calcifediol, ng/mL	32.55 ± 16.41	17.32 ± 7.82 [a]	12.61 ± 9.15 [a, b]	10.05 ± 10.11 [a, b, c]
Albumin, g/L	35.63 ± 3.34	35.56 ± 4.42	34.36 ± 2.21	34.55 ± 3.36
VDBP, μg/mL	338.22 ± 15.33	341.26 ± 18.22	333.56 ± 14.86	384.22 ± 15.42
eGFR, mL/min	100.34 ± 21.10	95.63 ± 18.35	90.75 ± 20.14	80.04 ± 24.27 [a, b, c]

Note: [a] $p < 0.05$ compared with control; [b] $p < 0.05$ compared with normal ACR; and [c] $p < 0.05$ compared with microalbuminuria.

Table 2. Analysis of β-cell function.

	Control	Diabetic patients		
		Normal ACR	Microalbuminuria	Macroalbuminuria
HOMA-IR	1.79 ± 0.78	4.38 ± 2.55 [a]	6.55 ± 2.67 [a, b]	7.89 ± 2.64 [a, b, c]
IAI	-4.22 ± 0.42	-4.41 ± 0.51	-4.47 ± 0.63	-4.45 ± 0.47
HOMA-BCF	4.88 ± 0.42	4.62 ± 0.24	4.37 ± 0.86	4.22 ± 0.66 [a]
Insulin secretion	0.92 ± 0.47	0.86 ± 0.56	0.57 ± 0.34	0.52 ± 0.49 [a, b]

Note: [a] $p < 0.05$ compared with control; [b] $p < 0.05$ compared with normal ACR; and [c] $p < 0.05$ compared with microalbuminuria.

Table 3. Analysis of cancer biomarkers.

	Control	Diabetic patients		
		Normal ACR	Microalbuminuria	Macroalbuminuria
AFP, ng/mL	1.5 ± 0.52	2.38 ± 0.99	3.1 ± 1.72 [a]	2.74 ± 0.91 [a]
CEA, ng/mL	2.28 ± 1.09	3.44 ± 1.51	4.69 ± 3.06 [a, b]	5.16 ± 2.18 [a]
CA199, U/mL	14.32 ± 11.56	20.75 ± 14.55	33.10 ± 25.21 [a, b]	34.90 ± 13.17 [a]
CA125, U/mL	7.60 ± 4.55	7.21 ± 2.66 [a]	10.45 ± 9.99 [a]	17.04 ± 12.47 [a]
CA153, U/mL	9.55 ± 2.17	11.18 ± 5.51	12.16 ± 5.35	12.41 ± 2.67
CA724, U/mL	1.22 ± 2.11	1.31 ± 2.43	2.24 ± 3.54	3.28 ± 4.16
Septin 9 methylation rate	1.3% (2/150)	0.8% (1/113)	7.8% (8/102) [a, b]	8.5% (9/105) [a, b]

Note: [a] $p < 0.05$ compared with control; and [b] $p < 0.05$ compared with normal ACR.

Table 4. Mean biomarker levels in T2DM patients positive or negative for septin 9 methylation.

	Positive	Negative	t	p
FPG, mmol/L	6.97 ± 3.10	6.41 ± 1.49	0.49	0.63
HbA1c, %	8.89 ± 2.65	6.76 ± 0.95	2.36	0.037
HOMA-IR,	5.04 ± 6.93	4.46 ± 5.91	0.19	0.84
Calcifediol, ng/mL	13.03 ± 4.15	19.80 ± 9.10	-2.12	0.49

Table 5. Correlation between serum calcifediol level and various clinical indices in T2DM patients.

	Before correction		After correction *	
	r	p	*r'*	p
BMI	-0.351	0.002	-	-
FPG	-0.241	0.043	-0.370	0.007
FINS	-0.091	0.014	-0.272	0.026
HbA1c	0.046	0.704	0.142	0.252
HDL-C	0.047	0.700	0.092	0.467
LDL-C	-0.022	0.854	-	-
TC	-0.108	0.368	-	-
TG	-0.121	0.314	-	-
SUA	-0.386	0.001	-0.421	0.002
SCr	-0.268	0.024	-0.191	0.170
BUN	-0.061	0.616	-0.085	0.544
HOMA-IR	-0.244	0.040	-	-
IAI	0.322	0.008	-0.004	0.967
HOMA-BCF	0.137	0.204	0.235	0.033
Insulin secretion	-0.087	0.344	0.187	0.028
AFP	-0.241	0.043	-0.196	0.112
CEA	-0.186	0.001	-0.200	0.015
CA199	-0.080	0.505	-0.024	0.850
CA125	-0.183	0.616	-0.134	0.324
CA153	-0.190	0.146	-0.155	0.254
CA724	-0.221	0.250	-0.127	0.158

Note: * After correction for BMI, TC, TG, LDL-C, and HOMA-I.

Table 6. Multivariate stepwise principal component regression analysis of CEA and relevant factors in T2DM patients.

	B	SE	β	t	p
Constant	-49.080	27.100	-	-1.811	0.000
Calcifediol	-0.303	0.332	-0.103	-0.914	0.003
HbA1c	4.541	2.092	0.272	2.171	0.034
SCr	0.136	0.044	0.480	3.087	0.003

β-cell function.

Indices of β-cell function

The β-cell function was then analyzed and compared. Our results showed that HOMA-IR in the healthy control group was significantly lower than in the T2DM groups. Moreover, HOMA-IR in the normoalbuminuria group was significantly lower than the microalbuminuria group, which was also significantly lower than the macroalbuminuria group (Table 2). Furthermore, the HOMA-BCF and insulin secretion in the healthy control group were significantly higher than the T2DM groups. HOMA-BCF and insulin secretion in the normoalbuminuria group were also significantly higher than the microalbuminuria group, which were significantly higher than the macroalbuminuria group. The correlation analysis indicated that the serum calcifediol level was significantly negatively correlated with SUA, SCr, and HOMA-IR, while positively correlated with IAI (Table 5). In addition, the serum calcifediol level was significantly negatively correlated with SUA, while positively correlated with HOMA-BCF and insulin secretion, after correction for BMI, LDL-C, TC, TG, and HOMA-IR.

CRC-associated tumor markers, ACR, and serum calcifediol level in T2DM

The CRC-associated tumor markers, ACR, and serum calcifediol level, in T2DM were investigated and analyzed. Our results showed that the serum levels of AFP, CEA, CA199, and CA125, as well as the rate of Septin9 methylation in the microalbuminuria and macroalbuminuria groups, were significantly higher than the healthy control group (Table 3). Moreover, among the T2DM patients, the rates of Septin9 methylation in the microalbuminuria and macroalbuminuria groups were significantly higher than the normoalbuminuria group. However, the rate of Septin9 methylation in the macroalbuminuria group was only slightly higher than the microalbuminuria group.

When T2DM patients were stratified according to the Septin9 methylation, the serum calcifediol level of negative patients was significantly lower compared with positive patients (Table 4). Serum calcifediol level was negatively correlated with the levels of AFP (uncorrected) and CEA (uncorrected and corrected) (Table 5). Our results from the multivariate stepwise principal component regression analysis (CEA as the dependent variable and calcifediol, HbA1c, and SCr as the independent variables) showed that, each of these three parameters represented an independent risk factor for elevated CEA (Table 6). Taken together, these results suggest that albuminuria and low levels of calcifediol are associated with higher risk of colorectal cancer (CRC).

DISCUSSION

In the present study, to investigate the significance of vitamin D in T2DM, the association between circulating calcifediol level and β-cell function and CRC-associated tumor markers were examined in a cohort of Chinese T2DM patients stratified according to albuminuria levels. Our results showed that the serum calcifediol level was independent of age, gender, BMI, or HDL-C, which was inversely associated with urine ACR. Moreover, lower serum calcifediol level was associated with greater insulin resistance, poorer β-cell function, and increased risk of CRC in T2DM patients with higher ACR. These findings suggest that vitamin D may have protective effects against albuminuria, β-cell dysfunction, and CRC in T2DM patients.

Several previous studies have already found that the vitamin D level is negatively associated with albuminuria in diabetic patients [5-7]. In fact, increasing evidence suggests that vitamin D has renoprotective effects in diabetic animals and humans [20,21]. Zhang et al. [21] have reported that the treatment with paricalcitol (vitamin D analog) could prevent albuminuria in diabetic mice by blocking the hyperglycemia-induced accumulation of renin and angiotensin-II. In addition, calcitriol has been shown to be able to prevent tubulointerstitial fibrosis by inhibiting the hyperglycemia-induced activation of transforming growth factor beta (TGF-β) [20]. Based on the data from the Third National Health and Nutrition Examination Survey (NHANES III), de Boer et al. [22] have shown that albuminuria (measured by ACR) is inversely associated with the calcifediol concentration, while calcifediol deficiency is independently linked to diabetes. Similarly, our results confirm that high serum calcifediol level was associated with low ACR, suggesting that vitamin D may have beneficial effects on renal functions in T2DM. However, further in-depth studies are still needed to investigate whether vitamin D insufficiency is the cause or result of albuminuria in these patients.

Hyperglycemia and hyperinsulinemia are major contributors to the systemic vascular permeability and endothelial dysfunction, which might change the hemodynamics in the kidney and the albuminuria levels in T2DM patients [23-25]. The association between the insulin resistance and albuminuria in T2DM has been investigated recently, however with inconsistent findings [5,9,26, 27]. In the present study, our results showed that the FINS, FPG, and HbA1c levels, as well as HOMA-IR in T2DM patients, were significantly higher than healthy individuals and were positively associated with ACR. In addition, HOMA-BCF and insulin secretion tended to be lower as the ACR increased. However, statistical significance was observed only between the diabetic macroalbuminuria and healthy control groups. These findings suggest that ACR may be an indicator of insulin resistance and β-cell dysfunction in T2DM patients. In fact, the association between β-cell function deterioration and increasing albuminuria level was previously

observed by Shin et al. [28] in T2DM patients and, more recently, by Fu et al. [29] in non-T2DM middle-aged and elderly Chinese individuals.

Our findings have shown that higher serum calcifediol is associated with lower ACR. Therefore, correlation analysis was conducted which confirmed that the serum calcifediol level was negatively correlated with SUA, SCr, and HOMA-IR. Higher serum calcifediol was associated with improved IAI, HOMA-BCF, and ISI, suggesting that calcifediol may be protective against the β-cell dysfunction in T2DM patients with albuminuria. However, studies by Cai et al. [5] and Fondjo et al. [30] have not indicated any significant association between the calcifediol level and β-cell function/insulin resistance. This contradiction may be attributed to different patient cohorts (e.g., size, ethnicity, geographic location, and medical history), models, or analysis methods used in these studies.

Earlier studies have indicated that vitamin D supplementation is an effective treatment for improving insulin resistance in T2DM [31,32]. However, recent reports have not found any significant changes in insulin sensitivity or secretion [33,34] when different dosages and duration periods were considered. Since the effects of vitamin D on insulin resistance, albuminuria, and disease chronology remain unclear, it is possible that vitamin D is only effective as an early prophylactic measure rather than as treatment after the disease onset. Therefore, further studies regarding the timing of vitamin D treatment will be required to better understand the effects of vitamin D on insulin resistance in T2DM patients with albuminuria.

CRC is a common diabetic complication, with increasing incidence in China [35]. Systematic reviews by Guraya [9] and Gonzalez et al. [8] have shown that the risk of CRC is positively associated with T2DM, and both hyperinsulinemia and hyperglycemia are implicated in the promotion of cell growth and inflammation, which contribute to increased cancer risks [36-38]. In line with this, our results showed that the CRC markers (such as AFP, CEA, CA199, CA125, and methylated Septin9) in T2DM patients were higher than healthy controls, which was significantly higher for the T2DM patients with albuminuria. These results suggest that T2DM patients with higher ACR may be at higher risk of developing CRC. Previous studies have shown that albuminuria is associated with cancer incidence in non-diabetic individuals, which is also an important risk factor for mortality in early CRC [10,11]. Although the mechanism underlying the role of albuminuria in CRC is still unknown, this phenomenon may be associated with increased glomerular permeability and decreased tubular reabsorption caused by elevated proinflammatory cytokines in the presence of neoplastic cells [39,40]. Extensive research on vitamin D in the past several years has revealed that the vitamin D/vitamin D receptor signaling has anti-tumor effects in CRC via gene regulation, which would induce cell differentiation and apoptosis [41,42] and inhibit cell proliferation [43] and metastasis [44]. Furthermore, studies on the extrarenal expression of hydroxyl vitamin D-1-hydroxylase in macrophages [45] and CD8+ T cells [46] suggest that these tumor-infiltrating cells may be able to generate calcitriol locally within the tumor, which would then exert their suppressive effects by binding to the vitamin D receptor on CRC cells.

In the present study, the serum calcifediol level of patients positive for Septin9 methylation was significantly lower than patients who were negative for Septin9 methylation. Septin9 is a tumor suppressor gene located on human chromosome 17, whose methylation would abrogate its tumor suppressive function and lead to uncontrolled cell growth and cancerous transformation. Consistent with the findings that higher calcifediol level is associated with lower risk for CRC [47,48], our results showed that the serum calcifediol level was negatively correlated with the AFP and CEA levels, while it was an independent risk factor for CEA. These findings suggest that the maintenance of high vitamin D level in T2DM patients, especially for those with albuminuria, may have potential protective effects against subsequent CRC development and progression. Of course, further investigation on the samples in subsequent follow-up studies will be required to confirm this speculation.

There are several limitations in this study. First, for these subjects, exposure to the sun had not been controlled. Although vitamin D-related preparation was considered as an exclusion criterion, outdoor activities may have differed among individuals or seasons, which might be a source of bias in this study. Therefore, subjects should be asked to provide a summary of their outdoor routine to help researchers better adjust the baseline vitamin D levels. Second, most biomarkers examined herein were measured from the blood and urine samples taken at a specific time, and their levels may differ depending on the time of day or the patients' conditions. As a result, additional and more direct tests, such as endoscopic biopsy and various imaging techniques, will help confirm the findings in patients during the subsequent follow-up visits. Third, this study only examined an elderly population of patients, while parallel examination of a younger population of similar T2DM patients may provide better insights into the patterns of the assessed variables over time.

CONCLUSION

Our study showed that the serum calcifediol level was inversely associated with the albuminuria levels in T2DM patients. Higher serum calcifediol level was correlated with better β-cell function, lower insulin resistance, and decreased risk of CRC. Therefore, appropriate high vitamin D level may protect against β-cell dysfunction and cancer development in T2DM patients with albuminuria. These results warrant further verification and assessments of vitamin D as a potential treat-

ment for diabetic complications.

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Declaration of Interest:
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References:

1. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. PLoS Med 2006;3:e442 (PMID: 17132052).

2. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care 2004;27:1047-53 (PMID: 15111519).

3. Mayer-Davis EJ, Lawrence JM, Dabelea D, et al. Incidence Trends of Type 1 and Type 2 Diabetes among Youths, 2002 - 2012. N Engl J Med 2017;376:1419-29 (PMID: 28402773).

4. Agarwal R. Vitamin D, proteinuria, diabetic nephropathy, and progression of CKD. Clin J Am Soc Nephrol 2009;4:1523-8 (PMID: 19478099).

5. Cai X, Hu Z, Chen L, Han X, Ji L. Analysis of the associations between vitamin D and albuminuria or beta-cell function in Chinese type 2 diabetes. Biomed Res Int 2014;2014:640909 (PMID: 4017846).

6. Suzuki A, Kotake M, Ono Y, et al. Hypovitaminosis D in type 2 diabetes mellitus: Association with microvascular complications and type of treatment. Endocr J 2006;53:503-10 (PMID: 16829706).

7. Zoppini G, Galletti A, Targher G, et al. Lower levels of 25-hydroxyvitamin D3 are associated with a higher prevalence of microvascular complications in patients with type 2 diabetes. BMJ Open Diabetes Res Care 2015;3:e000058 (PMID: 25932330).

8. Gonzalez N, Prieto I, Del Puerto-Nevado L, et al. 2017 update on the relationship between diabetes and colorectal cancer: epidemiology, potential molecular mechanisms and therapeutic implications. Oncotarget 2017;8:18456-85 (PMID: 28060743).

9. Guraya SY. Association of type 2 diabetes mellitus and the risk of colorectal cancer: A meta-analysis and systematic review. World J Gastroenterol 2015;21:6026-31 (PMID: 26019469).

10. Jorgensen L, Heuch I, Jenssen T, Jacobsen BK. Association of albuminuria and cancer incidence. J Am Soc Nephrol 2008;19:992-8 (PMID: 2386729).

11. Kim MJ, Kang YU, Kim CS, et al. Proteinuria as a risk factor for mortality in patients with colorectal cancer. Yonsei Med J 2013;54:1194-201 (PMID: 23918569).

12. Ness RA, Miller DD, Li W. The role of vitamin D in cancer prevention. Chinese journal of natural medicines 2015;13:481-97. https://www.sciencedirect.com/science/article/pii/S1875536415300431

13. Momeni A, Mirhosseini M, Kabiri M, Kheiri S. Effect of vitamin D on proteinuria in type 2 diabetic patients. J Nephropathol 2017;6:10-4 (PMID: 28042548).

14. Huang Y, Yu H, Lu J, et al. Oral supplementation with cholecalciferol 800 IU ameliorates albuminuria in Chinese type 2 diabetic patients with nephropathy. PLoS One 2012;7:e50510 (PMID: 23209764).

15. Pourhoseingholi MA. Increased burden of colorectal cancer in Asia. World J Gastrointest Oncol 2012;4:68-70 (PMID: 22532878).

16. Ganepola GA, Nizin J, Rutledge JR, Chang DH. Use of blood-based biomarkers for early diagnosis and surveillance of colorectal cancer. World J Gastrointest Oncol 2014;6:83-97 (PMID: 24734154).

17. Wasserkort R, Kalmar A, Valcz G, et al. Aberrant septin 9 DNA methylation in colorectal cancer is restricted to a single CpG island. BMC Cancer 2013;13:398 (PMID: 23988185).

18. deVos T, Tetzner R, Model F, et al. Circulating methylated SEPT9 DNA in plasma is a biomarker for colorectal cancer. Clin Chem 2009;55:1337-46 (PMID: 19406918).

19. Song L, Peng X, Li Y, et al. The SEPT9 gene methylation assay is capable of detecting colorectal adenoma in opportunistic screening. Epigenomics 2017;9:599-610 (PMID: 28470092).

20. Zhang Z, Sun L, Wang Y, et al. Renoprotective role of the vitamin D receptor in diabetic nephropathy. Kidney Int 2008;73:163-71 (PMID: 17928826).

21. Zhang Z, Zhang Y, Ning G, Deb DK, Kong J, Li YC. Combination therapy with AT1 blocker and vitamin D analog markedly ameliorates diabetic nephropathy: blockade of compensatory renin increase. Proc Natl Acad Sci USA 2008;105:15896-901 (PMID: 18838678).

22. de Boer IH, Ioannou GN, Kestenbaum B, Brunzell JD, Weiss NS. 25-Hydroxyvitamin D levels and albuminuria in the Third National Health and Nutrition Examination Survey (NHANES III). Am J Kidney Dis 2007;50:69-77 (PMID: 17591526).

23. Catalano C, Muscelli E, Quinones Galvan A, et al. Effect of insulin on systemic and renal handling of albumin in nondiabetic and NIDDM subjects. Diabetes 1997;46:868-75 (PMID: 9133557).

24. Hsu CC, Chang HY, Huang MC, et al. Association between insulin resistance and development of microalbuminuria in type 2 diabetes: a prospective cohort study. Diabetes Care 2011;34:982-7 (PMID: 21335369).

25. Tucker BJ, Anderson CM, Thies RS, Collins RC, Blantz RC. Glomerular hemodynamic alterations during acute hyperinsulinemia in normal and diabetic rats. Kidney Int 1992;42:1160-8 (PMID: 1453601).

26. Esteghamati A, Ashraf H, Nakhjavani M, Najafian B, Hamidi S, Abbasi M. Insulin resistance is an independent correlate of increased urine albumin excretion: a cross-sectional study in Iranian Type 2 diabetic patients. Diabet Med 2009;26:177-81 (PMID: 19236623).

27. Rizvi A, Varasteh B, Chen YD, Reaven GM. Lack of a relationship between urinary albumin excretion rate and insulin resistance in patients with non-insulin-dependent diabetes mellitus. Metabolism 1996;45:1062-4 (PMID: 8781291).

28. Shin SJ, Lee YJ, Hsaio PJ, et al. Relationships between beta-cell function and diabetic duration and albuminuria in type 2 diabetes mellitus. Pancreas 1997;14:192-8 (PMID: 9057193).

29. Fu S, Zhou S, Luo L, Ye P. Relationships of pancreatic beta-cell function with microalbuminuria and glomerular filtration rate in middle-aged and elderly population without type 2 diabetes mellitus: a Chinese community-based analysis. Clin Interv Aging 2017;12:753-7 (PMID: 28496313).

30. Fondjo LA, Owiredu W, Sakyi SA, et al. Vitamin D status and its association with insulin resistance among type 2 diabetics: A case-control study in Ghana. PLoS One 2017;12:e0175388 (PMID: 28423063).

31. Hu FB, Manson JE, Liu S, et al. Prospective study of adult onset diabetes mellitus (type 2) and risk of colorectal cancer in women. J Natl Cancer Inst 1999;91:542-7 (PMID: 10088625).

32. Talaei A, Mohamadi M, Adgi Z. The effect of vitamin D on insulin resistance in patients with type 2 diabetes. Diabetol Metab Syndr 2013;5:8 (PMID: 23443033).

33. Gulseth HL, Wium C, Angel K, Eriksen EF, Birkeland KI. Effects of Vitamin D Supplementation on Insulin Sensitivity and Insulin Secretion in Subjects with Type 2 Diabetes and Vitamin D Deficiency: A Randomized Controlled Trial. Diabetes Care 2017; 40:872-8 (PMID: 28468770).

34. Jamka M, Wozniewicz M, Jeszka J, Mardas M, Bogdanski P, Stelmach-Mardas M. The effect of vitamin D supplementation on insulin and glucose metabolism in overweight and obese individuals: systematic review with meta-analysis. Sci Rep 2015;5: 16142 (PMID: 26543018).

35. Zhang Y, Shi J, Huang H, Ren J, Li N, Dai M. [Burden of colorectal cancer in China]. Zhonghua Liu Xing Bing Xue Za Zhi 2015;36:709-14 (PMID: 26564699).

36. Ahmed RL, Schmitz KH, Anderson KE, Rosamond WD, Folsom AR. The metabolic syndrome and risk of incident colorectal cancer. Cancer 2006;107:28-36 (PMID: 16721800).

37. Lin Y, Berg AH, Iyengar P, et al. The hyperglycemia-induced inflammatory response in adipocytes: the role of reactive oxygen species. J Biol Chem 2005;280:4617-26 (PMID: 15536073).

38. Rinaldi S, Rohrmann S, Jenab M, et al. Glycosylated hemoglobin and risk of colorectal cancer in men and women, the European prospective investigation into cancer and nutrition. Cancer Epidemiol Biomarkers Prev 2008;17:3108-15 (PMID: 18990751).

39. Pedersen LM, Sorensen PG. Mediators of inflammation correlate with microalbuminuria in patients with non-Hodgkin's lymphoma. Br J Haematol 2003;121:275-9 (PMID: 12694249).

40. Pollock CA, Poronnik P. Albumin transport and processing by the proximal tubule: physiology and pathophysiology. Curr Opin Nephrol Hypertens 2007;16:359-64 (PMID: 17565279).

41. Díaz GD, Paraskeva C, Thomas MG, Binderup L, Hague A. Apoptosis is induced by the active metabolite of vitamin D3 and its analogue EB1089 in colorectal adenoma and carcinoma cells: possible implications for prevention and therapy. Cancer Res 2000;60:2304-12 (PMID: 10786699).

42. Vandewalle B, Wattez N, Lefebvre J. Effects of vitamin D3 derivatives on growth, differentiation and apoptosis in tumoral colonic HT 29 cells: possible implication of intracellular calcium. Cancer Lett 1995;97:99-106 (PMID: 7585485).

43. Scaglione-Sewell BA, Bissonnette M, Skarosi S, Abraham C, Brasitus TA. A vitamin D3 analog induces a G1-phase arrest in CaCo-2 cells by inhibiting cdk2 and cdk6: roles of cyclin E, p21Waf1, and p27Kip1. Endocrinology 2000;141:3931-9 (PMID: 11089522).

44. Evans SR, Shchepotin EI, Young H, Rochon J, Uskokovic M, Shchepotin IB. 1,25-dihydroxyvitamin D3 synthetic analogs inhibit spontaneous metastases in a 1,2-dimethylhydrazine-induced colon carcinogenesis model. Int J Oncol 2000;16:1249-54 (PMID: 10812003).

45. Adams JS, Hewison M. Extrarenal expression of the 25-hydroxyvitamin D-1-hydroxylase. Arch Biochem Biophys 2012;523:95-102 (PMID: 22446158).

46. Ooi JH, McDaniel KL, Weaver V, Cantorna MT. Murine CD8+ T cells but not macrophages express the vitamin D 1α-hydroxylase. J Nutr Biochem 2014;25:58-65 (PMID: 24314866).

47. Ekmekcioglu C, Haluza D, Kundi M. 25-Hydroxyvitamin D Status and Risk for Colorectal Cancer and Type 2 Diabetes Mellitus: A Systematic Review and Meta-Analysis of Epidemiological Studies. Int J Environ Res Public Health 2017;14 (PMID: 24314866).

48. Jacobs ET, Kohler LN, Kunihiro AG, Jurutka PW. Vitamin D and Colorectal, Breast, and Prostate Cancers: A Review of the Epidemiological Evidence. J Cancer 2016;7:232-40 (PMID: 26918035).