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ORIGINAL RESEARCH

Circulating Interleukin-22 Is a Biomarker for Newly Diagnosed Type 2 Diabetes Mellitus and Associated with Hypoglycemic Effect of Sitagliptin

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Purpose: Interleukin-22 (IL-22) has been demonstrated to be involved in the regulation of glucose metabolism, insulin resistance and inflammation response, which indicates that IL-22 might be associated with the occurrence and progression of diabetes. This study aimed to assess serum IL-22 levels in participants with type 2 diabetes mellitus (T2DM) and analyze the association between IL-22 levels and T2DM risk.

Methods: Serum IL-22 concentrations of recruited healthy participants (n=48), newly diagnosed T2DM participants (n=46), and T2DM participants receiving placebo (n=7) or dipeptidyl peptidase-4 inhibitors (DPP-4i) sitagliptin monotherapy (n=7) were measured using a commercial enzyme-linked immunosorbent assay (ELISA) kit. Mice fed a high-fat diet (HFD) were administered sitagliptin and evaluated for IL-22 and intestinal inflammation-related indicators.

Results: Serum IL-22 levels were higher in the T2DM group (127.16 ± 75.35) than in healthy controls (69.18 ± 32.83, $p < 0.001$), significantly negatively correlated with high-density lipoprotein cholesterol (HDL-C), and positively correlated with body mass index (BMI), glycosylated hemoglobin (HbA1c) and fasting plasma glucose (FPG), regardless of adjustment for sex and age. Multivariate logistic regression analysis showed that serum IL-22 levels were associated with the risk of T2DM (OR = 2.37, 95% CI = 1.27–4.42, $p = 0.007$). Additionally, sitagliptin treatment decreased the levels of IL-22 in the serum and colon tissues of T2DM participants and HFD mice. Moreover, intestinal inflammation was improved, and retinoid acid-related orphan receptor γt (RORγt, a marker of Th17 cells)- positive cells in the colon of HFD mice were decreased after sitagliptin treatment, which might be related to the reduction of IL-22.

Conclusion: Serum IL-22 is a significant independent risk factor for T2DM, implying that circulating IL-22 may be a predictive biomarker and therapeutic target for T2DM.

Keywords: IL-22, type 2 diabetes, sitagliptin, intestinal inflammation

Introduction

Type 2 Diabetes (T2DM) is a major global epidemiological event, and more than 500 million adults around the world are suffering from the disease [1] (*International Diabetes Federation Diabetes Atlas 2021. IDF Atlas 10th edition. Available at*: https://www.diabetesatlas.org). Various factors, such as genetics, diet, and the environment, are involved in the occurrence and development of T2DM. However, the etiology and pathogenesis of T2DM are not fully understood. Accumulating evidence has demonstrated that T2DM is accompanied by a chronic state of low-grade inflammation and an imbalance in the composition and function of intestinal flora.[2–4]

The dysbiosis of intestinal flora in individuals with obesity and T2DM is mainly manifested by an increase in the ratio of *Firmicutes* to *Bacteroidetes*, which impairs the integrity of the intestinal barrier and leads to the infiltration of lipopolysaccharide (LPS).[5] LPS, a cell wall component of gram-negative bacteria, can affect the ratio of Treg/Th17 cells to induce intestinal

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inflammation.[6] Upon entrance into circulation through the impaired intestinal barrier, some bacterial products and Th17 cells may induce chronic inflammation and metabolic disorders in metabolic tissues such as visceral adipose tissue and the liver.[5,7] A chronic inflammatory state in T2DM individuals has been reported to be associated with an increase in the percentage of circulating Th17 cells and a decrease in the percentage of Treg cells.[8] Moreover, studies have shown that the Th17 cells produced interleukin-22 (IL-22) was involved in the regulation of insulin resistance, as well as glucose metabolism through affecting inflammatory response. IL-22 promoted the production of IL-1β by activating the c-Jun pathway to induce pro-IL-1β transcription in macrophages, subsequently inducing insulin resistance.[9] Additionally, IL-22 treatment significantly inhibited the insulin-mediated glucose uptake in isolated rat soleus muscles.[10] In primary hepatocytes, IL-22 treatment reduced the phosphorylation level of Akt, a key protein kinase in the insulin signaling pathway.[10] Contrary to the aforementioned view, recent studies reported that IL-22 has protective roles in inflammation and diabetes. Zhang Peng et al found that administration of recombinant IL-22 improved liver steatosis, inflammation and insulin resistance in MASLD.[11] Similarly, it has been reported that targeting IL-22 in the pancreas and liver reduced hyperglycemia by improving β-cell function and insulin secretion.[12] These discrepancies may be attributed to differences in experimental models and the diversity of signaling pathways. These studies indicated that IL-22 was associated with insulin resistance, and might be involved in the pathogenesis of metabolic diseases such as obesity and diabetes.

Our previous study showed that the dipeptidyl peptidase-4 inhibitors (DPP-4i) sitagliptin, an oral antidiabetic agent, improved the dysbiosis of gut microbiota in T2DM individuals and high-fat diet (HFD) mice and induced a pattern shift of microbial metabolites, including short-chain fatty acids (SCFAs), branched-chain amino acids, and tryptophan derivatives, which might mediate glucose homeostasis.[13,14] Among the significantly increased metabolites, indole-3-acetic acid (IAA, a tryptophan derivative) has been reported to reduce the proportion of Th17 cells and increase the proportion of Treg cells in the lamina propria of the ileum in a mouse model of ankylosing spondylitis. Consequently, IAA reduced the production of pro-inflammatory factors and improved the intestinal barrier.[15] Likewise, sitagliptin has also been found to directly reduce the proportion of Th17 cells in the peripheral blood of individuals and mice with diabetes,[16,17] suggesting that sitagliptin might play a role in improving inflammation while lowering blood glucose levels. In fact, we initially observed that sitagliptin treatment reduced the number of retinoid acid-related orphan receptor γt (RORγt) (a marker of Th17 cells) -positive cells in colon tissues of HFD mice. Therefore, we speculated that Th17 produced IL-22 might be associated with the pathogenesis of T2DM and improvement of intestinal inflammation mediated by sitagliptin.

In the current study, to investigate the association between IL-22 and the risk of T2DM, we recruited a subgroup of our previous study including healthy controls and newly diagnosed T2DM participants. We first analyzed the association between circulating IL-22 levels and the risk of T2DM, and the hypoglycemic effect of DPP-4i sitagliptin in newly diagnosed T2DM individuals and HFD mice. Moreover, we assessed the relationship between IL-22 levels and intestinal inflammation in the HFD mice.

Materials and Methods

Healthy and T2DM Participants

This study enrolled ninety-four individuals. Individuals with T2DM were newly diagnosed based on the 1998 World Health Organization diagnostic criteria:[18] fasting plasma glucose (FPG) $\geq$ 7.0 mmol/L, 2-hour postprandial plasma glucose (2hPG) $\geq$ 11.1 mmol/L or both. The exclusion criteria were as follows: (i) presence of acute or chronic complications of diabetes; (ii) pregnancy; and (iii) history of chronic physical/mental diseases such as cancer, Alzheimer's disease, or Parkinson's disease. The experimental protocol was approved by the Ethics Committee of the Second Affiliated Hospital of the Army Medical University and was registered online (Clinical Trial Registry Number ChiCTR-ROC-17010719). Written informed consent was obtained from all the participants.

Sitagliptin treatment participants were recruited from our previous study.[13] Briefly, one group of newly diagnosed T2DM participants was treated with sitagliptin (n = 7), and the other group was treated with placebo (n = 7). Sitagliptin (Merck Sharp & Dohme) was administered at 100 mg/d. The detailed inclusion and exclusion criteria for T2DM were described in our previous study. Written informed consent was obtained from all the participants. The study was approved by the Ethics Committee of the Second Affiliated Hospital of the Army Medical University and was registered online (ChiCTR-OPC-17010757).

The current study involves secondary analysis of serum samples obtained from 94 participants from our previous studies,[14,19] and 14 participants from another clinical study.[13] Enzyme-linked immunosorbent assay (ELISA) of serum IL-22 was performed for each set of samples.

In the study, power calculation was performed using PASS software (version 15.0, NCSS, Silver Spring, Md) to ensure sufficient statistical power for detecting meaningful differences in the subgroup analysis. The calculation was based on a significance level (α) of 0.01, and a statistical power of 0.9. Based on these parameters, the required sample size for the healthy group and the T2DM group was estimated to be 40 participants, to achieve adequate power to detect the differences in IL-22 levels between subgroups.

Animal Study

C57BL/6 male mice were purchased from GemPharmatech Co., Ltd., China, and fed a normal diet (ND) or a HFD (60% fat, 20% protein, 20% carbohydrate (kcal/100 g), Research Diets, #D12492) for 14 weeks. HFD mice were divided into two groups according to the method of stratified randomization, to match weight and fasting blood glucose (FBG), and treated with 4 g/kg of sitagliptin or vehicle for the last 4 weeks during the HFD period. The animal experiment was conducted in strict adherence to the Guide for the Care and Use of Laboratory Animals, and in accordance with protocols approved by the Laboratory Animal Welfare and Ethics Committee of the Army Medical University (Approval No: AMUWEC20234810).

Measurement of Serum IL-22 Levels

The levels of cytokines (such as IL-22) might vary over time, and be influenced by biological rhythms, such as diurnal cycles, and differences in sampling time may lead to fluctuations in IL-22 measurement values. Therefore, for both human and mouse samples, we ensured an 8–12 hour fasting period before blood collection in the morning. Then, the blood samples were centrifuged at 3000 rpm for 15 minutes at 4°C to separate the serum, and immediately stored at −80 °C.

Serum IL-22 concentrations in T2DM and healthy participants were measured using a commercial ELISA kit according to the manufacturer's instructions (Human IL-22 ELISA kit, Proteintech Group, Inc., #KE00008). The detection sensitivity of the kit was 14.2 pg/mL. The intra- and inter-assay coefficients of variation (CV) were lower than 8.1%. All the samples were diluted 2 times before detection. No significant cross-reactivity or interference was observed during this assay.

Serum IL-22 concentrations in ND and HFD mice were measured using an ELISA kit (Mouse IL-22 ELISA kit, Proteintech Group, Inc., #KE10101). The detection sensitivity of this kit was 0.4 pg/mL. The intra-assay CV was lower than 2.7%, and the inter-assay CV was lower than 5.5%. No significant cross-reactivity or interference was observed during this assay.

Quantitative Real-Time PCR (qRT-PCR)

Total RNA was extracted from the colon tissues using RNAiso Plus (Takara, #9109). One microgram of total RNA was reverse-transcribed into cDNA using the PrimeScript RT Reagent Kit with gDNA Eraser (Takara, # RR047A). qRT-PCR was performed using the TB Green Premix (Takara, # RR820A) on a CFX Connect Real-Time System (Bio-Rad). The relative mRNA expression level was calculated using the following equation: Relative expression = $2^{-\text{(Ct (target gene)} - \text{Ct (reference gene))}}$. GAPDH was used as an internal reference gene for normalization. Primer sequences used are listed in Supplementary Table 1.

Hematoxylin-Eosin (H&E) and Immuno-Histochemical (IHC) Staining

Colon tissues from ND and HFD mice were collected using the Swiss-rolling method.[20] Briefly, the colon tissues were fixed in 4% paraformaldehyde, embedded in paraffin and sectioned. Tissue slides (4 μm) were deparaffinized in xylene, rehydrated with ethanol, and incubated for 10 min in 3% hydrogen peroxide to quench the endogenous peroxidase activity. For antigen retrieval, the slides were heated in citrate buffer. Then, the slides were incubated overnight at 4 °C with primary antibody. Nuclei were counterstained with hematoxylin. Lastly, DAB chromogen was detected using a commercial IHC staining kit (ZSGB-BIO, PV-6000). Anti-RORγ antibody (Abcam, #ab207082, 1:3000) was used as the primary antibody.

Statistical Analysis

All statistical analyses were conducted using SPSS software (IBM, Armonk, NY, version 26.0). The significance of differences between two groups was analyzed using Student's *t*-test, except for gender difference, which was analyzed using the chi-square test. To compare multiple groups, differences were analyzed using one-way ANOVA. Correlations between clinical variables and serum IL-22 levels were estimated with Spearman correlation and partial correlation analysis. Binary logistic regression analysis was conducted to explore the association between serum IL-22 levels and the risk of T2DM. Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic effectiveness of circulating IL-22 levels in T2DM. A p value < 0.05 was defined as statistically significant.

Results

The Serum Level of IL-22 Was Higher in T2DM Participants

To investigate the association between serum IL-22 levels and T2DM, 48 healthy controls and 46 T2DM participants were included in this study. The main clinical characteristics of the enrolled individuals were presented in Table 1. Sex and age were matched between healthy and T2DM groups. There were no significant differences in total cholesterol (TC), aspartate aminotransferase (AST), serum creatinine (Scr), blood urea nitrogen (BUN), or uric acid (UA) between the two groups. Compared to the healthy controls, the T2DM group had higher levels of hemoglobin A1c (HbA1c), FPG, triglyceride (TG), low-density lipoprotein-cholesterol (LDL-C), alanine aminotransferase (ALT) and γ-glutamyl transferase (GGT), while the levels of high-density lipoprotein-cholesterol (HDL-C) were significantly lower in the T2DM group ($p < 0.001$). As expected, the T2DM group exhibited a significant increase in serum IL-22 levels (69.18±32.83 vs 127.16±75.35, $p < 0.001$, Table 1 and Figure 1A).

In addition, we analyzed the correlation between IL-22 levels and metabolic parameters. As shown in Table 2, serum IL-22 concentrations were positively correlated with body mass index (BMI), HbA1c, FPG, LDL-C and ALT, and negatively correlated with HDL-C (Table 2). After adjusting for sex and age, the negative correlation between IL-22 levels and HDL-C and the positive correlations between IL-22 and BMI, HbA1c and FPG remained significant ($p < 0.05$, Table 2 and Figure 1B–D).

Table 1 Clinical Characteristics of Enrolled Healthy and T2DM Participants

Variables	Health (n=48)	T2DM (n=46)	*p* value
Sex (men/women)	18/30	25/21	0.101
Age (year)	49.42 ± 11.70	52.91 ± 10.43	0.130
BMI (kg/m^2)	22.56 ± 2.63	25.54 ± 2.36	<0.001
HbA1c (%)	5.6 ± 0.32	8.3 ± 1.92	<0.001
HbA1c (mmol/mol)	37 ± 3.49	68 ± 21.03	<0.001
FPG (mmol/L)	5.03 ± 0.49	7.84 ± 2.58	<0.001
TG (mmol/L)	1.18 ± 0.45	1.84 ± 0.92	<0.001
TC (mmol/L)	4.93 ± 0.68	4.86 ± 1.10	0.809
HDL-C (mmol/L)	1.45 ± 0.30	1.11 ± 0.22	<0.001
LDL-C (mmol/L)	2.52 ± 0.41	3.16 ± 0.83	<0.001
ALT (IU/L)	21.07 ± 14.51	29.08 ± 17.04	0.016
AST (IU/L)	20.66 ± 7.08	22.96 ± 9.26	0.179
GGT (IU/L)	22.97 ± 11.84	37.47 ± 29.12	0.003
Scr (μmol/L)	61.59 ± 12.11	65.91 ± 12.84	0.097
BUN (mmol/L)	5.60 ± 1.61	5.43 ± 1.21	0.556
UA (μmmol/L)	304.76 ± 74.54	308.41 ± 97.06	0.927
IL-22 (pg/mL)	69.18 ± 32.83	127.16 ± 75.35	<0.001

Notes: Data are presented as mean ± SD. *p* value was calculated by *t*-test.
Abbreviations: BMI, body mass index; HbA1c, glycosylated hemoglobin; FPG, fasting plasma glucose; TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyl transferase; Scr, Serum creatinine; BUN, blood urea nitrogen; UA, serum uric acid.

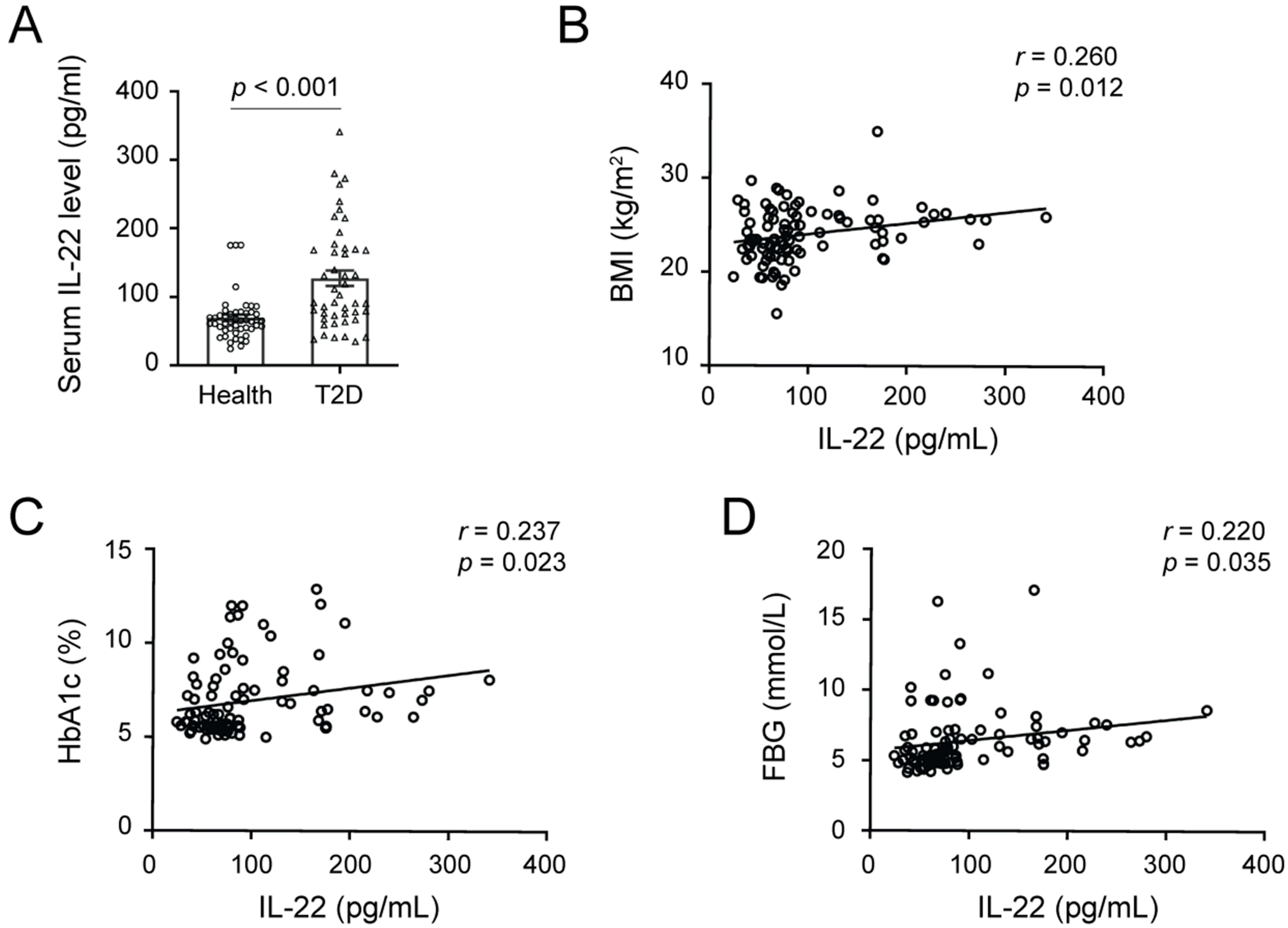

Figure 1 The serum IL-22 levels were increased in T2DM participants. (**A**) The levels of IL-22 in the serum of healthy controls and newly diagnosed T2DM participants. Data are presented as means ± S.E.M. *p* value was calculated by *t* test. (**B-D**). The correlations between serum IL-22 and BMI (**B**), HbA1c (**C**), and FBG (**D**). Data were analyzed by partial correlation test (sex and age adjusted), n=94.

Serum IL-22 Level Was Positively Associated With T2DM Risk

To further confirm the association between serum IL-22 levels and the risk of T2DM, the enrolled participants were divided into four groups according to the quartile of IL-22 levels (quartile 1, <58.00; quartile 2, 58.00–75.04; quartile 3, 75.04–121.85; quartile 4, >121.85pg/mL). Univariate regression analysis showed that serum IL-22 levels and several metabolic characteristics, including BMI, TG, HDL-C, LDL-C, ALT, and GGT levels, were significantly associated with T2DM risk (Table 3). After adjusting for these characteristics, the results of the multivariate analysis showed that serum IL-22 level was a significant independent risk factor for T2DM (OR = 2.37, 95% CI = 1.27–4.42, p = 0.007).

Additionally, the ability of circulating IL-22 to predict T2DM was tested by ROC curve and AUC calculation. As shown in Supplementary Figure 1, circulating IL-22 levels exhibited a relatively good ability to differentiate T2DM from healthy individuals (AUC (95% CI) = 0.767 (0.667–0.866), $p < 0.001$). These results suggest that circulating IL-22 might be a potential diagnostic biomarker of T2DM.

Sitagliptin Treatment Decreased the Level of IL-22 and Improves Intestinal Inflammation

Next, to further investigate whether the hypoglycemic agent DPP-4i could change IL-22 levels in the clinical setting, we measured serum IL-22 concentrations in another group of newly diagnosed T2DM participants who either received sitagliptin or did not receive any antidiabetic agents. This subset of individuals was enrolled in our previous study. The detection showed that sitagliptin monotherapy induced a decreasing trend in IL-22 levels, although there was no

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Table 2 Correlations Between Clinical Characteristics and Serum IL-22 Levels in the Participants

Variables	*r*	*p* value	*r*	*p* value
			Sex and Age Adjusted	
Sex (men/women)	−0.035	0.740	–	–
Age (year)	−0.027	0.797	–	–
BMI (kg/m2)	0.259	0.012	0.260	0.012
HbA1c (%)	0.332	0.001	0.237	0.023
FBG (mmol/L)	0.394	<0.001	0.220	0.035
TG (mmol/L)	0.174	0.094	0.125	0.235
TC (mmol/L)	−0.075	0.475	−0.144	0.170
HDL-C (mmol/L)	−0.291	0.004	−0.309	0.003
LDL-C (mmol/L)	0.300	0.003	0.154	0.143
ALT (IU/L)	0.276	0.007	0.145	0.169
AST (IU/L)	0.115	0.271	0.086	0.417
GGT (IU/L)	0.191	0.065	0.061	0.562
Scr (μmol/L)	0.168	0.105	0.215	0.040
BUN (mmol/L)	−0.105	0.314	−0.064	0.542
UA (μmmol/L)	0.175	0.091	0.305	0.003

Notes: Correlations between IL-22 concentrations and clinical characteristics were analyzed by Spearman analysis or sex- and age-adjusted partial correlation tests (n = 94).

Abbreviations: r, correlation coefficient; BMI, body mass index; HbA1c, glycosylated hemoglobin; FBG, fasting blood glucose; TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyl transferase; Cr, Serum creatinine; BUN, blood urea nitrogen; UA, serum uric acid.

Table 3 The Association Between Serum IL-22 Levels and T2DM Risk

Risk Factors	Univariate		Multivariate	
	OR (95% CI)	*p* value	OR (95% CI)	*p* value
IL-22(groups)	2.68 (1.70,4.21)	< 0.001	2.37 (1.27,4.42)	**0.007**
Sex	1.98 (0.87,4.52)	0.103	–	–
Age	1.03 (0.99,1.07)	0.133	–	–
BMI	1.70 (1.34,2.15)	< 0.001	1.25 (0.92,1.70)	0.161
TG	5.05 (2.16,11.84)	< 0.001	1.23 (0.38,4.02)	0.730
TC	0.95 (0.60,1.48)	0.805	–	–
HDL-C	0.004 (0.00,0.05)	< 0.001	0.007 (0.00,0.25)	0.006
LDL-C	5.67 (2.31,13.92)	< 0.001	5.06 (1.40,18.30)	0.014
ALT	1.04 (1.01,1.07)	0.025	0.972 (0.92,1.03)	0.334
AST	1.04 (0.98,1.09)	0.185	–	–
GGT	1.05 (1.02,1.09)	0.004	1.03 (0.98,1.10)	0.265
Cr	1.03 (0.10,1.06)	0.100	–	–
BUN	0.92 (0.69,1.22)	0.552	–	–
UA	1.00 (1.00,1.01)	0.926	–	–

Notes: Binary logistic regression analysis was conducted to analyze the association between the clinical characteristics and the risk of T2DM. Health, n=46; T2DM, n=48.

Abbreviations: OR, odds ratio; CI, confidence interval.

statistically significant difference (130.1 vs 103.1, $p = 0.070$, Figure 2A). Consistently, serum levels of IL-22 were higher in HFD mice than in ND mice. After sitagliptin treatment, IL-22 levels in the serum of HFD mice (HFD-Sit) significantly decreased (Figure 2B). Moreover, the expression level of IL-22 in the colon tissues of HFD mice was also higher than

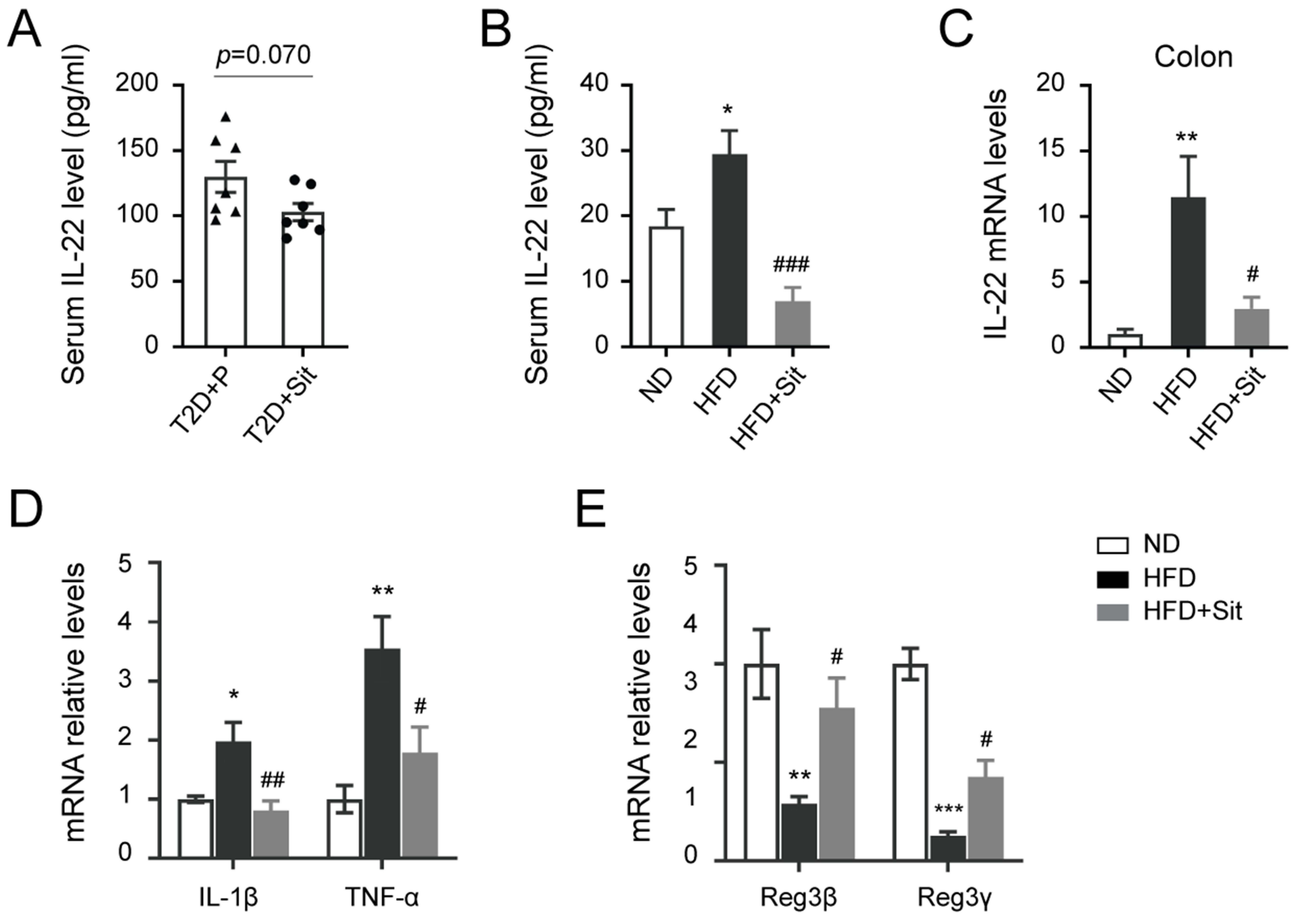

Figure 2 The levels of IL-22 were decreased in T2DM participants and HFD mice after sitagliptin treatment. (**A**) The serum levels of IL-22 in T2DM participants administrated with or without sitagliptin (Sit). n=7. (**B**) The serum levels of IL-22 in ND mice, and HFD mice treated with or without sitagliptin. (**C-E**) The mRNA expression levels of IL-22 (**C**), inflammatory factors (**D**), and antimicrobial peptides (**E**) in the colon tissues of ND, HFD and HFD+Sit mice. All data in the bar blot are expressed as the mean ± S.E.M. B-E, n=5 mice per group, *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ versus the ND group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ versus the HFD group.

that in ND mice, and was distinctly decreased by sitagliptin (Figure 2C). These results suggest that IL-22 levels were associated with the hypoglycemic effect of DPP-4i sitagliptin.

Meanwhile, the expression levels of pro-inflammatory factors (IL-1β and TNF-α) were significantly reduced, and the expression levels of antimicrobial peptides (Reg3β and Reg3γ) were obviously increased in the colon tissues of sitagliptin-treated HFD mice (Figure 2D and E), indicating that sitagliptin treatment attenuated HFD-induced intestinal inflammation. In addition, compared to ND mice, H&E staining of the colon tissue sections of HFD mice revealed detached mucosal epithelial cells and atrophied crypts (Figure 3A). The number of lymphoid nodules in the lamina propria of the colon was increased, some of which were fused in the HFD group. Increased inflammatory cell infiltration in the lamina propria and submucosa was observed in the colon tissues of HFD mice. In contrast, sitagliptin treatment attenuated the inflammatory status induced by HFD. Moreover, IHC staining for RORγt (a marker of Th17 cells)[21] was performed to assess the number of Th17 cells. As shown in Figure 3B, the number of RORγt-positive cells in the colon tissues of HFD mice was higher than that in ND mice, however, the number of specific cells decreased in sitagliptin (Sit) treated mice (HFD+Sit), indicating that sitagliptin may reduce IL-22 levels by decreasing the number of Th17 cells in the colon tissues of HFD mice. Taken together, these results suggest that IL-22 might be associated with improvement in intestinal inflammation mediated by the hypoglycemic agent sitagliptin.

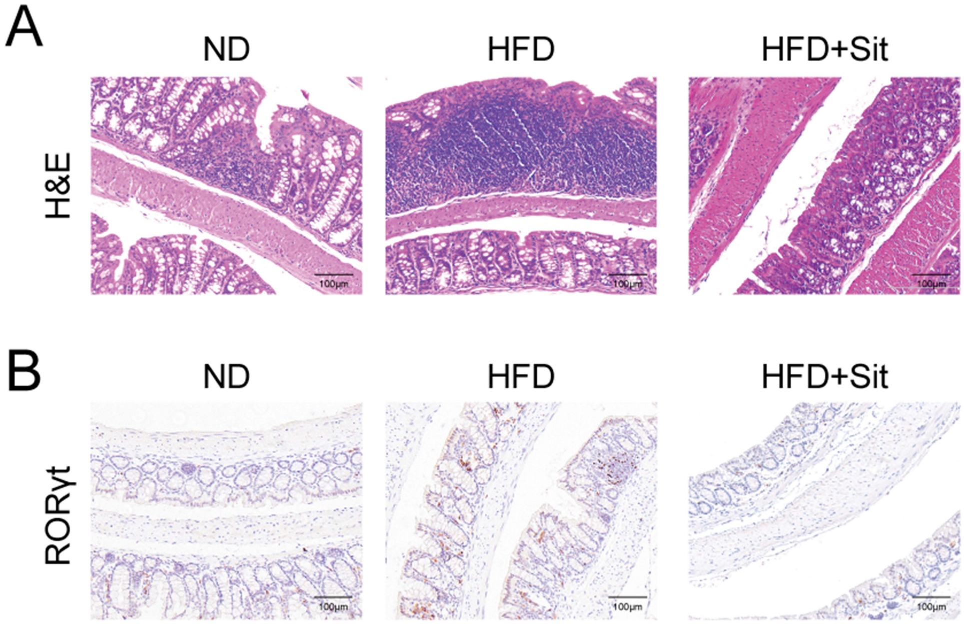

Figure 3 RORγT positive cells were decreased in the colon of HFD mice after sitagliptin treatment. (**A**) Representative images of H&E staining of colon tissue sections. (**B**) IHC staining of RORγt in the colon tissues of ND, HFD and HFD+Sit mice. Scale bar, 100μm.

Discussion

Accumulating evidence supports that chronic inflammation contributes to the onset and progression of diabetes.[22,23] In this study, we observed that circulating IL-22 levels were significantly increased in newly diagnosed T2DM participants. Moreover, the regression analysis indicated that serum level of IL-22 was an independent risk factor for T2DM. Additionally, ROC analysis suggested that circulating IL-22 levels exhibited a relatively good ability to differentiate T2DM patients from healthy individuals. These results suggested that circulating IL-22 might be a potential diagnostic biomarker for T2DM.

Th17/IL-22 axis has been implicated in the pathogenesis of intestinal inflammatory responses and metabolic diseases.[24,25] It has been reported that the increase of Th17 cells in peripheral blood and adipose tissues is related to BMI, serum HDL levels and insulin resistance.[8,26,27] Th17 cells that secrete IL-22 have been identified to be involved in the regulation of insulin resistance, as well as glucose metabolism by affecting the inflammatory response.[10] Additionally, high serum levels of IL-22 were positively associated with several cardiometabolic risk factors.[28] However, a mouse model of obesity revealed the beneficial effects of IL-22 treatment on inflammation and insulin sensitivity.[29] Several clinical studies have found that IL-22 levels in peripheral blood might be associated with diabetes, but the conclusions are inconsistent.[30,31] These contradictory results might be attributed to differences in the course of diabetes, clinical treatment, sample numbers of enrolled participants, and so on. In this study, we recruited participants with newly diagnosed T2DM participants, and excluded the influence of different treatments. Nevertheless, prospective studies and fundamental research are needed to determine the role of IL-22 in the pathogenesis of T2DM.

In a previous study, we demonstrated that treatment with the hypoglycemic agent DPP-4i improved glucose homeostasis, accompanied by alterations in the composition of the gut microbiota and metabolites in HFD mice.[13] HFD and gut microbiota, as well as their interactions, could induce chronic low-grade inflammation in the intestine.[32,33] Dysbiosis of gut microbiota induced an increase in the levels of endotoxins and pathogens in the intestine. These toxic substances promote intestinal macrophages to release pro-inflammatory factors such as TNF-α and histamine, resulting in an inflammatory response. Chronic inflammation exacerbated intestinal permeability, allowing bacterial LPS to pass through the intestinal barrier and affecting the secretion spectrum of intestinal hormones.[34,35] These changes are crucial to the occurrence and development of obesity and diabetes. On the other hand, *Lactobacillus* produced indole-3-lactic acid (ILA) was demonstrated to protect against intestinal inflammation and promoted the expression of key bacterial enzymes implicated in tryptophan metabolism, leading to the synthesis of other indole derivatives, including IAA and indole-

3-propionic acid (IPA).[36] Indole, acting as a ligand of aryl hydrocarbon receptor (AhR), has been reported to inhibit inflammation to improve hepatic steatosis and insulin resistance.[37,38] Our previous metabolomic data showed that the relative content of IAA was significantly increased in the feces of sitagliptin-treated HFD mice.[13] IAA has been reported to reduce the proportion of Th17 cells,[15] and inhibit LPS-stimulated production of pro-inflammatory cytokines in macrophages.[39] That is to say, gut microbiota contributes to inflammation and metabolic dysfunction might be associated with the bacterial metabolites or produced enzymes. Additionally, in the current study, sitagliptin treatment decreased the number of RORγt-positive cells, and reduced IL-22 levels in the colon tissues of HFD mice. These results suggest that sitagliptin might improve inflammation status by increasing IAA to reduce the production of IL-22, which might be an additional benefit of DPP-4i like sitagliptin, suggesting a broader therapeutic utility. Further fundamental studies are required to clarify this issue.

While the current study has some limitations that need to be pointed out. First, our study used a cross-sectional design, so the causality between IL-22 levels and T2DM could not be clarified. Second, due to the small sample size of the T2DM group that received sitagliptin monotherapy, the correlation between IL-22 levels and clinical treatment with sitagliptin could not be determined, which highlights the need for studies with larger sample sizes or longitudinal studies to confirm our findings in the future. On the other hand, mouse experiments were performed and observed sitagliptin treatment reduced IL-22 levels in the serum and colon tissues, which supports the findings of clinical trials, to some extent. While, further molecular experiments should be performed to explore the role and molecular mechanism of DPP-4i/IAA/IL-22 in regulating chronic inflammation.

In conclusion, our study demonstrated that serum levels of IL-22 were significantly increased in newly diagnosed T2DM participants when compared to healthy participants and were positively correlated with the risk of T2DM. Moreover, DPP-4i sitagliptin treatment obviously decreased IL-22 levels accompanied by improving intestinal inflammation. Thus, circulating IL-22 may be a biomarker for T2DM diagnosis and a potential therapeutic target for T2DM.

Data Sharing Statement

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

Ethics Approval and Consent to Participate

The study was approved by the Ethics Committee of the Second Affiliated Hospital of Army Medical University and registered online (Clinical trial registry number ChiCTR-ROC-17010719 and ChiCTR-OPC-17010757). Informed written consent was obtained from all the participants, and the research was conducted in accordance with the principles outlined in the Declaration of Helsinki.

Consent for Publication

Consent for publication was obtained from all study participants.

Author Contributions

All authors contributed to data acquisition, data analysis, drafting or revising the article, have agreed on the journal to which the article will be submitted, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

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Disclosure

All authors declare no conflicts of interest.

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