The Variation and Correlation of Serum Adiponectin, Nesfatin-1, IL-6 and TNF- α Levels in Prediabetes

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Research Article

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Abstract

Background: The variation and correlation among adiponectin, nesfatin-1, TNF- α and IL-6, which involve in the process from healthy to prediabetes and diabetes have not been elucidated. This study aims to investigate the roles of them in this process.

Methods: 72 T2DM, 75 prediabetes and 72 healthy individuals were enrolled and serum adiponectin, nesfatin-1, TNF- α and IL-6 were tested with appropriate kit, and primary data were analyzed with correct method.

Results: serum levels of each factor in prediabetes were between T2DM and healthy, and significant differences were found among them. TNF- α and nesfatin-1 levels in T2DM obviously different compared to prediabetes or healthy; IL-6 and adiponectin levels in the healthy group were significantly changed in contrast to prediabetes or T2DM. Correlation analysis found that in prediabetes, adiponectin was positively correlated with TNF- α ($R=0.2939$, $P=0.0105$) and IL-6 ($R=0.3918$, $P=0.0005$), and the relationship greatly strengthened when prediabetes accompanied by insulin resistant (TNF- α : $R=0.7732$, $P<0.0001$, IL-6: $R=0.6663$, $P=0.0005$). We also demonstrated that declined adiponectin ($OR=6.238$, $P=0.019$), nesfatin-1 ($OR=2.812$, $P=0.01$) and elevated TNF- α ($OR=5.541$, $P=0.001$) were risk factors for prediabetes towards diabetes.

Conclusions: this research proved significant variations of adiponectin, nesfatin-1, IL-6 and TNF- α levels in the healthy, prediabetes and T2DM, suggesting a slow and gradual change during the progression of healthy condition toward diabetes via prediabetes.

Background

Prediabetes occurs before type 2 diabetes mellitus (T2DM) and is a high-risk state for diabetes development. It is predicted that there will be 470 million prediabetes until 2030^[1]. Until 2019, there were nearly 463 million T2DM patients, and this number will increase to 700 million in 2045^[2]. T2DM is characterized by insulin resistance and islet β cell dysfunction. Some studies believed that β cell dysfunction started even before the impaired glucose tolerance^[3, 4]. It has been proved that inflammation in islet of T2DM patient is due to immune cell infiltration^[5], amyloid deposition^[6], cell death^[7] and fibrosis^[8, 9]. Tumor necrosis factor α (TNF- α) and interleukin 6 (IL-6) as proinflammatory factors secreted by M1 macrophage^[10] could impair insulin secretion through degrading insulin receptor substrate^[11]. Elevated TNF- α and IL-6 levels in comparison to healthy controls were observed in T2DM serum^[12]. However, the variation of TNF- α and IL-6 levels in prediabetes and their comparison with T2DM and the healthy group have not been disclosed.

Adiponectin was secreted by adipocytes and first found as a protein similar to complement 1q^[13]. Adiponectin is a 30-kDa monomeric glycoprotein^[14], which exerts extensive physiological effects such as improving insulin sensitivity^[15], promoting anti-inflammatory^[16] and enhancing vascular protection^[17].

Several case-control studies^[18, 19] and meta-analysis^[20] proved that decreased circulating adiponectin in serum was associated with higher T2DM risk. Nesfatin-1 is a satiety molecule found in the hypothalamus by Oh-I in 2006^[21]. Nesfatin-1 has been found could improve insulin resistance and glucose uptake through Akt/AMPK/TORC2 pathway in an animal study^[22]. Recently, some studies investigated the role of nesfatin-1 in the development of T2DM confirmed the variation of the serum nesfatin-1 levels in T2DM patients, but their conclusions were not consistent^[23, 24]. A meta-analysis figured out that serum nesfatin-1 level elevated in newly diagnosed T2DM but decreased in those patients who received antidiabetic treatment^[25]. However, few studies have focused on the change of nesfatin-1 levels in prediabetes.

So far, the variation of adiponectin, nesfatin-1, IL-6 and TNF- α levels in T2DM has been extensively explored, which identified elevated levels of IL-6 and TNF- α and reduction of adiponectin and nesfatin-1 levels. However, the change of these factors in prediabetes and their correlation has not been delineated. In this study, we aimed to probe into the differences and connections of adiponectin, nesfatin-1, IL-6 and TNF- α levels in prediabetes, T2DM and the normal participants, in order to better understand the roles of these factors in the development of prediabetes and T2DM.

Materials And Methods

Subjects

From August 2020 to February 2021, 75 prediabetes (58.56 ± 9.73 years), 72 T2DM (58.07 ± 9.60 years) consecutive participants who attended Xiangya Hospital of Central South University (Hunan province, China) and met the criteria of American Diabetes Association^[26] were recruited. Meanwhile, 72 (56.88 ± 6.59 years) age-sex-matched healthy volunteers were introduced in this study. All participants were given informed consent and this study was permitted by the ethics committee of Xiangya Hospital of Central South University (No 202009119).

Materials

5ml venous blood was extracted for biochemical analysis from each participant who fasted at least 8 h. Serum was collected by centrifuging the blood at 3600 rpm for 5 min and stored at -20°C until detection.

Methods

All tests were conducted at Xiangya Hospital of Central South University. Serum levels of nesfatin-1, IL-6 and TNF- α were determined by enzyme linked immunosorbent assay (ELISA) kit (Kete Biological Technology, Jiangsu, China), while adiponectin was measured by latex enhanced immune turbidimetry (Guangdong Uniten Biotechnology, Guangdong, China). Insulin was detected with chemiluminescent immunoassay (Beckman Coulter, CA, USA). Other parameters such as total triglyceride (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), total bilirubin (TB), direct bilirubin (DB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bile acid (TBA), serum creatinine (Scr), fasting blood-glucose (FBG) and 2-hour blood glucose

(2hBG) were detected on AU5800 chemistry analyzer (Beckman Coulter, CA, USA) with the appropriate kits. Hemoglobin A1c(HbA1c) was quantified using high performance liquid chromatography (ARKRAY, Kyoto, Japan). Indexes related to insulin were calculated according the following formulars: Homeostasis model assessment of insulin resistance (HOMA-IR) = insulin ($\mu\text{U/ml}$) * FBG (mmol/l)/22.5; homeostasis model assessment of beta-cell function (HOMA- β) = 20 * insulin ($\mu\text{U/ml}$)/ (FBG (mmol/L)-3.5); homeostasis model assessment of insulin sensitivity (HOMA-IS) = 22.5/insulin ($\mu\text{U/ml}$) * FBG (mmol/l) [27], and eGFR (MDRD) [$\text{ml}\cdot\text{min}^{-1}\cdot(1.73\text{ m}^2)^{-1}$] = $30849\cdot[\text{Scr}(\mu\text{mol/L})]^{-1.154}\cdot(\text{age})^{-0.203}$ (multiply by 0.742 for women) [28].

Statistical analysis

Statistical analysis was carried out using SPSS version 26 for Windows (SPSS Inc., IL, USA). The results were presented as mean $\pm$ standard deviation for continuous variables and as numbers for categorical variables. Differences among groups or subgroups were calculated with ANOVA. Correlation between nesfatin-1 and other indexes was analyzed with Pearson. Influence factors involving the process of developing prediabetes into T2DM, or interfering with HOMA-IR and HbA1c were determined by logistic regression analysis. $P < 0.05$ (two-tailed) was regarded as statistically significant.

Results

General Characteristics

In total, 219 participants were enrolled in this study and their anthropometry characteristics and clinical paraments were listed at Table 1. There were not noteworthy differences of gender composition, age and body mass index (BMI) among the three groups. Results showed their concentrations in prediabetes vs in T2DM vs in the healthy were: adiponectin (5.12 ± 2.31 vs 4.79 ± 1.92 vs 5.74 ± 1.91 $\mu\text{g/ml}$), nesfatin-1 (802.58 ± 570.13 vs 489.85 ± 349.92 vs 1034.54 ± 1174.07 pg/ml), TNF- α (382.81 ± 308.63 vs 663.95 ± 693.19 vs 342.06 ± 160.89 pg/ml) and IL-6 (29.69 ± 29.41 vs 38.46 ± 60.48 vs 16.55 ± 15.29 pg/ml), indicating that the serum levels of each factor in prediabetes were between in T2DM and the healthy group. Variation of the levels among them were statistically analyzed. As shown in Fig. 1a, serum adiponectin levels in T2DM or prediabetes were significantly decreased compared with the healthy while no apparent difference was observed between T2DM and prediabetes. Serum IL-6 levels in T2DM or prediabetes were dramatically increased in contrast to the healthy, while no significant variation was displayed between the prediabetes and T2DM (Fig. 1b). Profound elevation of TNF- α in T2DM was found compared with prediabetes or the healthy group, but it showed insignificant rise when compared prediabetes with healthy (Fig. 1c). Nesfatin-1 levels in T2DM were dramatically declined in contrast to the healthy or prediabetes, while no significant reduction was presented when compare prediabetes with the healthy (Fig. 1d).

Table 1
Anthropometric and Clinical characteristics of the study subjects in different groups

Parameter	T2DM	Prediabetes	Healthy
Male/female	50/29	49/26	47/25
Age (years)	58.07 ± 9.60	58.56 ± 9.73	56.88 ± 6.59
BMI (Kg/m ²)	22.48 ± 3.26	23.76 ± 5.45	22.87 ± 4.36
IL-6 (pg/ml)	38.46 ± 22.23	29.69 ± 29.41*	16.55 ± 15.29
TNF-α (pg/ml)	663.95 ± 693.19**#	382.81 ± 308.63	342.06 ± 160.89
Nesfatin-1 (pg/ml)	489.85 ± 349.92**#	802.58 ± 570.13	1034.54 ± 1174.07
Adiponectin (μg/ml)	4.79 ± 1.92*	5.12 ± 2.31	5.71 ± 1.91
Insulin (μU/ml)	12.16 ± 10.02*	11.14 ± 12.14*	5.89 ± 2.77
HOMA-IR	4.85 ± 5.57**#	3.19 ± 3.52**	1.37 ± 0.70
HOMA-β	66.05 ± 79.55	76.43 ± 81.45	68.57 ± 40.71
HOMA-IS	0.44 ± 0.67**	0.58 ± 0.40**	0.93 ± 0.56
TG(μmol/L)	2.31 ± 2.73*	2.14 ± 1.83*	1.25 ± 0.41
TC (μmol/L)	4.84 ± 0.73#	5.52 ± 1.05*	5.11 ± 1.63
LDL-C (mmol/L)	3.21 ± 0.99#	3.50 ± 0.71*	2.99 ± 0.55
HDL-C (mmol/L)	1.14 ± 0.32**#	1.28 ± 0.30	1.35 ± 0.26 [!]
HDL/LDL	0.42 ± 0.48	0.38 ± 0.14	0.46 ± 0.11
TB (μmol/L)	11.43 ± 5.35	11.89 ± 6.09	10.83 ± 3.19
DB (μmol/L)	5.58 ± 2.72	5.76 ± 2.84	5.45 ± 1.55
ALT (U/L)	27.95 ± 32.12	31.39 ± 24.52*	21.77 ± 8.75
AST (U/L)	25.44 ± 16.71	27.87 ± 12.22	23.83 ± 5.46

Abbreviations: BMI: body mass index; IL-6: interleukin 6; TNF-α: tumor necrosis factor α; HOMA-IR: homeostasis model assessment of insulin resistance; HOMA-β: homeostasis model assessment of β cell; HOMA-IS: homeostasis model assessment of insulin sensitivity; TG: triglyceride; TC: total cholesterol; LDL-C: low density lipoprotein-cholesterol; HDL-C: high density lipoprotein-cholesterol; TB: total bilirubin; DB: direct bilirubin; ALT: alanine aminotransferase; AST: aspartate aminotransferase; TBA: total bile acid; Scr: serum creatinine; eGFR: estimated glomerular filtration; HbA1c: glycosylated hemoglobin; FPG: fasting blood glucose; 2hBG: 2 hours blood glucose. #: vs prediabetes $P < 0.05$; *: vs healthy $P < 0.05$; **: vs healthy $P < 0.01$.

Parameter	T2DM	Prediabetes	Healthy
TBA ($\mu\text{mol/L}$)	5.03 ± 5.12	4.62 ± 3.78	3.64 ± 2.67
Scr ($\mu\text{mol/L}$)	$100.67 \pm 70.87^{* \#}$	79.94 ± 13.16	74.83 ± 12.49
eGFR ($\text{ml} \cdot \text{min}^{-1} \cdot (1.73 \text{ m}^2)^{-1}$)	74.55 ± 23.20	80.93 ± 26.13	79.43 ± 9.69
HbA1c (%)	$7.81 \pm 1.94^{* \#}$	$5.95 \pm 0.26^*$	5.49 ± 0.35
FPG (mmol/l)	$8.78 \pm 3.14^{* \#}$	$6.41 \pm 0.34^*$	5.28 ± 0.43
2hBG (mmol/l)	$14.85 \pm 5.40^{* \#}$	9.06 ± 1.38	5.99 ± 0.95
Abbreviations: BMI: body mass index; IL-6: interleukin 6; TNF- α : tumor necrosis factor α ; HOMA-IR: homeostasis model assessment of insulin resistance; HOMA- β : homeostasis model assessment of β cell; HOMA-IS: homeostasis model assessment of insulin sensitivity; TG: triglyceride; TC: total cholesterol; LDL-C: low density lipoprotein-cholesterol; HDL-C: high density lipoprotein-cholesterol; TB: total bilirubin; DB: direct bilirubin; ALT: alanine aminotransferase; AST: aspartate aminotransferase; TBA: total bile acid; Scr: serum creatinine; eGFR: estimated glomerular filtration; HbA1c: glycosylated hemoglobin; FPG: fasting blood glucose; 2hBG: 2 hours blood glucose. #: vs prediabetes $P < 0.05$; *: vs healthy $P < 0.05$, **: vs healthy $P < 0.01$.			

Characteristics of subgroups divided by HOMA-IR

To further investigate the roles of nesfatin-1, adiponectin, IL-6 and TNF- α in the development of T2DM, T2DM and prediabetes participants were distributed to four subgroups with cut-off 2.8 of HOMA-IR^[29], which included 29 T2DM patients with HOMA-IR < 2.8 (A), 43 T2DM patients with HOMA-IR > 2.8 (B), 52 prediabetes with HOMA-IR < 2.8 (C) and 23 prediabetes with HOMA-IR > 2.8 (D). Characteristics of these subgroups were listed at the supplementary table1. Nesfatin-1 levels in subgroup D (1028.15 ± 839.70 pg/ml) were significantly increased compared with subgroup A (420.80 ± 289.70 pg/ml), B (536.41 ± 381.48 pg/ml) and C (702.81 ± 366.59 pg/ml). Meanwhile nesfatin-1 serum levels in C subgroups outstandingly increased compared to A subgroup (Fig. 2a). TNF- α levels in subgroup A (648.53 ± 719.06 pg/ml) and B (674.35 ± 683.62 pg/ml) were outstandingly upregulated compared to subgroup C (364.19 ± 308.69 pg/ml) and D (424.92 ± 311.17 pg/ml) (Fig. 2b). Adiponectin in subgroup D (6.08 ± 3.20 $\mu\text{g/ml}$) significantly higher than subgroup and B (4.48 ± 1.66 $\mu\text{g/ml}$) and C (4.70 ± 1.65 $\mu\text{g/ml}$), but no difference between A (5.25 ± 2.20 $\mu\text{g/ml}$) and D (Fig. 2c). IL-6 levels in A (43.92 ± 22.23 pg/ml) subgroup obviously elevated in comparison to C (29.02 ± 19.02 pg/ml) or D (31.21 ± 14.95 pg/ml) subgroup but except B (34.78 ± 14.38 pg/ml) subgroup (Fig. 2d).

Correlations between adiponectin and other indexes

To further investigate the relationships among adiponectin, IL-6, TNF- α , correlations between adiponectin and other indexes were analyzed, the results were shown on Table 2. As described, serum levels of adiponectin in prediabetes were significantly correlated to IL-6 ($R = 0.3918$, $P = 0.0005$), TNF- α ($R = 0.2939$, $P = 0.0105$), insulin ($R = -0.3068$, $P = -0.0074$), HbA1c ($R = -0.3404$, $P = 0.0048$), HOMA-IR ($R = 0.2904$, $P =$

0.0115), and HOMA- β ($R = 0.3354$, $P = 0.0033$). The correlation between adiponectin and nesfatin-1 was slight ($R = -0.0146$, $P = 0.901$).

Table 2
Correlations between adiponectin and other indexes

Parameters	IL-6	TNF- α	Insulin	HbA1c	HOMA-IR	HOMA- β
Total participants						
R	0.1777	0.05	0.001	-0.1297	-0.062	0.060
<i>P</i> (two-tailed)	0.0084	0.46	0.987	0.080	0.356	0.373
Significant	**					
T2DM						
R	0.2597	0.068	-0.2198	0.012	0.2023	0.101
<i>P</i> (two-tailed)	0.0276	0.57	0.0636	0.923	0.088	0.3995
Significant	*					
Prediabetes						
R	0.3918	0.2939	-0.3068	-0.3404	0.2904	0.3354
<i>P</i> (two-tailed)	0.0005	0.0105	0.0074	0.0048	0.0115	0.0033
Significant	***	*	***	**	*	**
Healthy						
R	0.036	0.058	-0.2790	0.0117	-0.1961	0.3037
<i>P</i> (two-tailed)	0.7635	0.6282	0.0176	0.9393	0.0988	0.0095
Significant			*	**		
*: <i>P</i> < 0.05, **: <i>P</i> < 0.01, ***: <i>P</i> < 0.001						

Correlations between adiponectin and other indexes among subgroups divided by HOMA-IR

Adiponectin levels in the subgroups of prediabetes with HOMA-IR > 2.8 were found to be strongly correlated with IL-6 ($R = 0.6663$, $P = 0.005$) and TNF- α ($R = 0.7732$, $P < 0.001$) but in the other three subgroups, there was no such correlation (Table 3). Moreover, serum adiponectin in the subgroup of prediabetes with HOMA > 2.8 has powerful relevance to HDL-C ($R = 0.6891$, $P = 0.0003$), HDL-C/LDL-C ($R = 0.7005$, $P = 0.0002$) or TG ($R = -0.4263$, $P = 0.0425$), and in T2DM with HOMA-IR > 2.8 subgroup, such association was also presented between adiponectin and HDL-C: ($R = 0.3591$, $P = 0.0195$) or HDL-C/LDL-C: ($R = 0.3327$, $P = 0.0313$). Additionally, serum adiponectin in the subgroup of prediabetes with HOMA-IR

< 2.8 was found to be significantly related to TG ($R=-0.3016$, $P=0.0298$), LDL-C ($R=-0.3359$, $P=0.0149$) or HDL-C/LDL-C ($R=0.3813$, $P=0.0058$).

Table 3
Correlation of adiponectin with other indexes of subgroups

Parameters	IL-6	TNF-α	TG	LDL-C	HDL-C	HDL-C/LDL-C
T2DM HOMA-IR < 2.8						
R	0.4069	0.2902	-0.1225	-0.0810	0.1192	0.1803
<i>P</i> (two-tailed)	0.0285	0.1267	0.5267	0.6761	0.5379	0.3493
Significant	*					
T2DM HOMA-IR > 2.8						
R	-0.0162	-0.1271	0.0787	-0.1050	0.3591	0.3327
<i>P</i> (two-tailed)	0.9177	0.4167	0.6205	0.5083	0.0195	0.0313
Significant					*	*
Prediabetes HOMA-IR < 2.8						
R	-0.0008	-0.1074	-0.3016	-0.3359	0.1718	0.3813
<i>P</i> (two-tailed)	0.9957	0.4486	0.0298	0.0149	0.2232	0.0058
Significant			*	*	**	
Prediabetes HOMA-IR > 2.8						
R	0.6663	0.7732	-0.4263	-0.1837	0.6891	0.7005
<i>P</i> (two-tailed)	0.0005	< 0.0001	0.0425	0.4013	0.0003	0.0002
Significant	***	***	*	***		***
*: <i>P</i> < 0.05, **: <i>P</i> < 0.01, ***: <i>P</i> < 0.001						

Factors involved in the development of prediabetes toward T2DM

Risk factors which may affect prediabetes toward T2DM were assessed by logistic regression. Suffering from T2DM was introduced as the dependent variable, while the independent variables were IL-6, TNF- α , adiponectin, nesfatin-1, HOMA-IR, TG, TC, LDL-C, HDL-C, HDL-C/LDL-C, TB, DB, TBA, eGFR, HbA1c, FBG and 2hBG. The outcome in Table 4 disclosed that TNF- α (OR = 5.541, $P=0.001$), HOMA-IR (OR = 8.168, $P < 0.001$), TG (OR = 3.277, $P=0.024$), eGFR (OR = 13.655, $P=0.027$) and 2hBG (OR = 56.802, $P=0.002$) were the risk factors which may contribute to the development of prediabetes into T2DM, whereas insulin

(OR = 0.157, $P = 0.016$), TC (OR = 0.325, $P = 0.022$) and TB (OR = 0.110, $P = 0.012$) were protective factors which may impede the process.

Table 4
influence factors involved in the process from prediabetes to T2DM

Variable	B	S _b	Wald χ^2	P	OR	95%CI
TNF- α	1.712	0.505	11.517	0.001	5.541	2.061 ~ 14.894
Insulin	-1.849	0.766	5.834	0.016	0.157	0.035 ~ 0.706
HOMA-IR	2.100	0.555	14.323	0.000	8.168	2.753 ~ 24.238
TG	1.187	0.526	5.087	0.024	3.277	1.168 ~ 9.190
TC	-1.124	0.491	5.241	0.022	0.325	0.124 ~ 0.851
TB	-2.211	0.878	6.346	0.012	0.110	0.020 ~ 0.612
DB	1.256	0.692	3.295	0.069	3.511	0.905 ~ 13.621
eGFR	2.614	1.182	4.889	0.027	13.655	1.346 ~ 138.55
2hBG	4.040	1.299	9.674	0.002	56.802	4.455 ~ 724.282
Constant	-5.740	1.518	14.305	0.000	0.003	/

We further evaluated the influence factors of HOMA-IR and HbA1c by logistic regression. HOMA-IR and HbA1c were respectively set as dependent variables, and IL-6, TNF- α , adiponectin, nesfatin-1, TG, TC, LDL-C, HDL-C, HDL-C/LDL-C, TB, DB, TBA, eGFR, FBG and 2hBG were input as independent variables.

As exhibited in supplement Table 2, declined serum nesfatin-1 levels increased the risk of HOMA-IR (OR = 2.812, $P = 0.010$), while high FBG (OR = 4.082, $P = 0.006$), HbA1c (OR = 2.523, $P = 0.078$) levels improved the risk of HOMA-IR. Elevated ALT (OR = 0.151, $P = 0.018$) could protect the body from HOMA-IR. In addition, downregulated adiponectin (OR = 6.238, $P = 0.019$), elevated TB (OR = 12.382, $P = 0.041$), FBG (OR = 9.839, $P < 0.001$) and 2hBG (OR = 3.454, $P = 0.027$) could positively connect to HbA1c, but escalated DB (OR = 0.168, $P = 0.047$) levels seemed to be a negative factor to HbA1c (supplement Table 3).

Discussion

In this study, we firstly revealed the differences of adiponectin, nesfatin-1, IL-6 and TNF- α between prediabetes and T2DM or healthy individuals in Chinese population. Our results demonstrated that adiponectin levels in prediabetes were distinctly lower than that in the healthy, while no significant difference was observed when compared to T2DM, which was consistent with the consequences of previous studies^[30,31]. Another research reported significant reduction of adiponectin in impaired fasting glucose group compared with hyperglycemic group^[31]. Others proved mildly lower nesfatin-1 level in impaired fasting glycemia and impaired glucose tolerance in contrast to the healthy group^[23]. The

variation of the satiety molecule nesfatin-1 level in prediabetes hasn't been widely probed, despite a study found slightly low nesfatin-1 levels in impaired fasting glycemia and impaired glucose tolerance compared to the healthy [23]. Our data revealed that nesfatin-1 levels in prediabetes were obviously higher than that in T2DM but no significant difference when compared to the healthy, which is the first delineation. In our study, remarkably increased IL-6 levels were detected in prediabetes in contrast to the healthy but that levels insignificantly dropped when compared to T2DM. Upadhyaya et al. reported that serum IL-6 levels in impaired fasting glucose group was dramatically higher than normoglycemic group and mildly lower than hyperglycemic group [31]. It has been reported that IL-6 protein expression at sections of pancreas and isolated islets from prediabetes were significantly upregulated compared to the normal persons in a recent study, where the prediabetes criteria only included impaired fasting glucose and HbA1c [32]. Here we added indexes of FBG, 2hFBG and HbA1c into the prediabetes criteria to make the evidences more sufficient.

Based on the experimental evidences that TNF- α concentration in prediabetes was significantly lower than in T2DM but mildly higher than in the healthy, Lainampetch et al. proved that TNF- α promoted developing prediabetes into T2DM [33]. In agreement with their findings, we also found declined TNF- α level in prediabetes when compared to T2DM and moderate elevation when compared to the healthy. Correlation test in our study uncovered that TNF- α levels were strongly relevant to adiponectin in prediabetes ($R = 0.2939$, $P = 0.0105$).

Our logistic regression analysis indicated that elevated TNF- α , HOMA-IR, TG, TC, TB and 2hBG and reduced insulin and eGFR were risk factors for facilitating the progression of pre-diabetic condition to diabetes. Recent studies revealed that combination of insulin resistance and compensatory hyperinsulinemia could give rise to dyslipidemia, which characterized by high levels of TG, TC, LDL-C and reduction of HDL-C concentration in serum [34, 35]. To better understand the roles of nesfatin-1, adiponectin, IL-6 and TNF- α in the development of prediabetes and T2DM, we introduced influence factors of HOMA-IR and HbA1c in the present study due to insulin resistant and hyper insulinemia have major impacts on prediabetes evolvement.

Our results suggested that falling nesfatin-1 levels was a risk factor for HOMA-IR rising ($OR = 2.812$, $P = 0.01$) while reduced adiponectin level raised the risk of HbA1c elevation ($OR = 6.238$, $P = 0.019$). Many studies focused on nesfatin-1 and insulin resistant, decreased serum nesfatin-1 levels of insulin resistant associated disease such as polycystic ovarian syndrome [36], non-alcoholic fatty liver disease [37] had covered. An in vivo study demonstrated that injecting nesfatin-1 into the third cerebral ventricle of high-fat diet-fed rats contributed to promoting insulin-induced glucose uptake and insulin signaling through upregulating the phosphorylation levels of insulin receptors [22]. Nesfatin-1 was also found to be augmented in gastric cancer and exert decent role for diagnosing gastric cancer ($AUC = 0.857$, $95\%CI (0.769-0.946)$) with a threshold of 1.075 ng/ml [38]. There is growing evidence on nesfatin-1's versatility, but its receptor remains unknown.

In this study, adiponectin presented a significant positive correlation with TNF- α ($R = 0.2939$, $P = 0.0105$) or IL-6 ($R = 0.3918$, $P = 0.0005$) in prediabetes, and their relationship was notably enhanced in prediabetes with HOMA-IR (TNF- α : $R = 0.7732$, $P < 0.0001$, IL-6: $R = 0.6663$, $P = 0.0005$). As an endocrine organ, adipose tissue secretes numerous cytokines including TNF- α , IL-6, visfatin, leptin, and adiponectin. Adiponectin is an antiinflammation adipocytokine and macrophage is the primary target of adiponectin's anti-inflammation effect^[39]. Adiponectin inhibits the differentiation of myeloid progenitor cells, regulates the function of macrophage and decreases expression of Toll-like receptor 4^[40]. Moreover, adiponectin suppresses the proinflammatory M1 macrophage activation and elevates the activation and proliferation of M2 macrophage, as well as modulates macrophage polarization from M1 phenotype towards M2 cells^[41, 42]. It has been demonstrated that knock-out adiponectin in mice resulted in upregulates expression of M1 macrophage markers such as TNF- α , IL-6 and monocyte chemoattractant protein-1 and downregulation of M2 macrophage including arginase 1, macrophage galactose N-acetyl-galactosamine specific lectin-1 and IL-10^[42]. It is worth noting that adiponectin appeared to have proinflammatory effects according to a few researches^[43, 44]. Adiponectin induced human macrophage and T-cell differentiation to proinflammatory phenotype which resembles M1 other than M2^[43], and in human adipose tissue and placenta, adiponectin promoted the release of TNF- α and IL-6 through NF- κ B and ERK signaling^[44]. In addition, it has been proven that TNF- α could damage adiponectin multimerization and consequently impairs adiponectin secretion by altering disulfide bond modification in the endoplasmic reticulum^[45]. Collectively, adiponectin could function as an anti-inflammatory factor or proinflammatory molecule by inhibiting the polarization of macrophage M1^[43], or eliciting the secretion of TNF- α and IL-6 in human adipose tissues^[45]. It can be inferred that adiponectin participates in the progression of prediabetes toward diabetes and changes its roles of being anti-inflammatory or proinflammatory in response to different disease condition.

In summary, this research revealed that serum adiponectin, nesfatin-1, IL-6 and TNF- α levels in prediabetes were obviously change compared with T2DM or healthy individuals. We also found that adiponectin exerted its anti-inflammatory function in prediabetes phase, suggesting a significant but slow and gradual change during the progression of healthy condition toward diabetes via pre-diabetes.

This study also exists some limitations. Firstly, the research included only the Han nationality participants, thus racial differences should be noticed when apply the conclusion to other populations. Secondly, the size of the participants in this study is relatively small and more cases are needed to verify the variation of adiponectin, nesfatin-1, TNF- α and IL-6 in prediabetes and diabetes, as well as to make the conclusion more convincible. Thirdly, the cut-off HOMA-IR value of Chinese adults are vacant, studies are needed to definite the value. More importantly, the mechanisms of how the adiponectin, nesfatin-1, TNF- α and IL-6 factors are involved in the progression of prediabetes to diabetes are worth our in-depth exploration.

List Of Abbreviations

2hBG:2-hour blood glucose

ALT: alanine aminotransferase

AST: aspartate aminotransferase

DB: direct bilirubin

eGFR: estimated glomerular filtration rate

ELISA: enzyme linked immunosorbent assay

FBG: fasting blood-glucose

HbA1c: hemoglobin A1c

HDL-C: high density lipoprotein cholesterol

HOMA-IR: homeostasis model assessment of insulin resistance

HOMA-IS: homeostasis model assessment of insulin sensitivity

HOMA- β : homeostasis model assessment of beta-cell function

IL-6: interleukin 6

LDL-C: low density lipoprotein cholesterol

Scr: serum creatinine

T2DM: type 2 diabetes mellitus

TB: total bilirubin

TBA: total bile acid

TC: total cholesterol

TG: total triglyceride

TNF- α : tumor necrosis factor α

Declarations

Ethics approval and consent to participate

All participants were given informed consent and this study was permitted by the ethics committee of Xiangya Hospital of Central South University (No 202009119).

Consent for publication

Written informed consent for publication was obtained from all participants.

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests.

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Authors' Contributions

KH wrote the main manuscript text, YL and YM prepared figure1-2, JW and HL prepared Table 1-4. BY amend the first draft and approved the final version. All authors reviewed the manuscript.

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Figures

Figure 1

Comparison of serum levels of adiponectin, IL-6, TNF- α and nesfatin-1 among T2DM, prediabetes and the healthy. (a) Adiponectin levels in T2DM significantly decreased compared with healthy other than prediabetes, while no apparent difference was observed between T2DM and prediabetes. (b) IL-6 levels in T2DM or prediabetes were dramatically increased in contrast to the healthy, while no significant variation was displayed between the prediabetes and T2DM. (c) Profound elevation of TNF- α in T2DM was found compared with prediabetes or the healthy group, but it showed insignificant rise when compared prediabetes with healthy. (d) Nesfatin-1 levels in T2DM were dramatically declined in contrast to the healthy or prediabetes, while no significant reduction was presented when compare prediabetes with the healthy. #: vs Prediabetes $P < 0.05$; *: vs Healthy $P < 0.05$, **:vs Healthy $P < 0.01$.

Figure 2

Comparison of serum levels of adiponectin, IL-6, TNF-α and nesfatin-1 among the HOMA-IR subgroups. (a) Nesfatin-1 levels in subgroup D were significantly increased compared with other subgroups, nesfatin-1 levels in C subgroup also significantly elevated in comparison to A subgroup; (b) TNF-α levels in subgroup A, B were outstandingly upregulated compared to subgroup C or D. (c) Adiponectin levels in D subgroup obviously increased than B or C subgroup. (d) IL-6 levels in A subgroup significantly higher compared with C or D subgroup. *: vs Prediabetes with HOMA-IR under 2.8 $P < 0.05$; **: vs Prediabetes with HOMA-IR under 2.8 $P < 0.01$; #: vs Prediabetes with HOMA-IR above 2.8 $P < 0.01$; ##: vs Prediabetes with HOMA-IR above 2.8 $P < 0.01$; ###: vs Prediabetes with HOMA-IR above 2.8 $P < 0.001$.

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