Soluble OX40 is significantly increased in the serum of overweight and obese patients with type 2 diabetes

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Abstract

Object: Our study mainly aimed to explore the presence of sOX40 in the peripheral serum of overweight and obese patients.

Methods: 60 overweight and obese patients admitted to the endocrinology department of the first affiliated hospital of Soochow University were recruited into the study and divided into control group (25 participants) and T2DM group (35 participants) according to the World Health Organization diagnostic criteria. The differences of general statistics, biochemical parameters, islet function and sOX40 were compared among the two groups. The associations between HbA_{1c} and other parameters and the influences of various parameters on the severity of hyperglycemia were analyzed.

Results: Serum levels of OX40 was significantly higher in T2DM group compared with control group ($p < 0.05$). Pearson correlation analysis showed that HbA_{1c} was positively correlated to sOX40 and FBG and negatively correlated to BMI, 2hINS, FCP and 2hCP ($P < 0.05$). Multiple linear regression analysis showed significant positive influences of sOX40 and FBG on hyperglycemia in overweight and obese patients ($P < 0.05$).

Conclusion: Hyperglycemia in overweight and obese patients increased with sOX40. Higher sOX40 was the independent risk factors for hyperglycemia in overweight and obese patients.

Introduction

Obesity is a chronic metabolic disease caused by body fat accumulation and weight gain, due to excessive food intake or metabolic disorders in the body. It is closely related to the occurrence of insulin resistance, type 2 diabetes, hypertension, cardiovascular and cerebrovascular diseases^[1-2]. Most obese patients exist insulin resistance and will develop diabetes over time, which have more complications and larger harm with hyperglycemia^[3]. Therefore, it is very important for obese patients to lose weight, improve insulin resistance and prevent the occurrence of diabetes. There are a large number studies demonstrated that chronic low-grade inflammation of adipose tissue is a key role in the occurrence of insulin resistance in obese patients. Chronic low-grade inflammation of adipose tissue is mainly caused by abnormal activation of T lymphocytes, B lymphocytes and macrophages, especially the continuous activation of CD4⁺ T cells is particularly critical^[4-5]. OX40/OX40L, as an important costimulatory molecule, plays an important role in the development and progression in the activation of CD4⁺ T cells^[6]. Soluble OX40, which is thought to be cleaved from membrane OX40 by photolytic enzyme, is less studied in obese patients currently. Therefore, this study mainly aimed to explored the presence of sOX40 in the peripheral serum of overweight and obese patients and its effect on the blood glucose.

Methods

Patients and controls

The study enrolled 25 patients with overweight and obese (control group), and 35 overweight and obese with type 2 diabetes (T2DM group). All the subjects are age- and sex- matched. Clinical characteristics for the patients are presented in Table 1. Using the Working Group on Obesity in China (WGOC) criteria^[7], Normal BMI was defined as derived measures between 18.5-24 kg/m², obesity defined as a BMI $\geq$ 28 kg/m² and overweight defined as a BMI $\geq$ 24 kg/m² and $\leq$ 28 kg/m². The diagnostic criteria for T2DM were according to the World Health Organization (WHO) diagnostic criteria^[8]. All the patients are newly diagnosed, with no history of medication. In this study, blood samples were collected after overnight fasting from patients at the Endocrinology Department in the first Affiliated Hospital of Soochow University between 2019 and 2020. Prior to commencing this study, the approval from the Ethics Reviews Board of the first Affiliated Hospital of Soochow University was granted.

Table 1
Clinic and biochemical characteristics of the patients

	control group (n=25)	T2DM group (n=35)	P value
BMI(kg/m ²)	30.52± 0.46	28.84±0.93	0.019
FBG(mmol/l)	5.14± 0.07	8.90±0.49	<0.001
HbA _{1c} (%)	8.43± 0.13	10.76±0.38	<0.001
FINS(pmol/l)	18.52±2.32	15.32±1.27	0.111
2hINS(pmol/l)	101.70±14.13	31.23±4.39	<0.001
FCP(ng/ml)	2.64±0.24	1.96±0.21	0.023
2hCP(ng/ml)	9.07±0.63	5.26±0.48	<0.001
TC(mmol/l)	4.98± 0.12	5.04±0.17	0.921
TG (mmol/l)	1.92± 0.18	2.16±0.18	0.432
LDL-C (mmol/l)	2.86± 0.12	3.26±0.17	0.212
HDL-C(mmol/l)	1.02± 0.03	0.93±0.04	0.212
UA(mmol/l)	398.21±17.39	375.90±18.28	0.517
Body fat(kg)	30.66±1.11	31.24±2.62	0.870
Body fat rate(%)	35.58±0.89	35.17±1.66	0.819
HOMA-IR	4.32±0.62	5.93±0.53	0.045
^a P<0.05 compared with the control group; ^b P<0.01 compared with the control group; BMI: Body mass index; FPG: Fasting plasma glucose; HbA _{1c} : Glycosylated hemoglobin; FINS: Fasting insulin; 2hINS: insulin after 2h meal; FCP: Fasting C-peptide; 2hCP: C-peptide after 2h meal; HOMA-IR: Homeostasis assessment of insulin resistance; UA: Uric acid; TC: Total cholesterol; TG: Triglyceride; LDL-C: Low-density lipoprotein cholesterol; HDL-C: High-density lipoprotein cholesterol;			

Experiment methods

Blood samples from an antecubital vein were collected in a quiet state in the morning after an overnight fast of 12 hours. The blood samples were placed at room temperature for 30 minutes, and then partly centrifuged with 3500r/min for 10 minutes to extract the serums. Glycosylated hemoglobin (HbA_{1c}) was determined with high performance liquid chromatography (HLC-723G8, TOSOH Company, Japan). The analyses of biochemical parameters including fasting plasma glucose (FPG), uric acid (UA), total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were completed by an automatic biochemical analyzer (7600, HITACHI Company, Japan). Hormones including fasting insulin (FINS), fasting c-peptide (FCP), insulin after 2h meal and c-

peptide after 2h meal were analyzed with chemiluminescent immunoassay (AIA-2000ST, TOSOH Company, Japan). Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as FPG multiplied by FINS then divided by 22.5.

Serum OX40 concentration was quantified by enzyme-linked immune- sorbent assay (ELISA). The samples were centrifuged at 1500 rpm for 10 min, and the cell-free sera were stored at -20°C for the ELISA assay. The levels of sOX40 in the sera were determined in single well using the sOX40 ELISA system prepared in Institute of Clinical Immunology Research Laboratory of Jiangsu Province, Suzhou, China, previously [9].

The human body composition analyzer (manufactured by Inbody) is used to measure the human body fat and body fat rate according to the body's electrical impedance characteristics and changes through the multi-frequency bioelectrical impedance method (BIA)^[10]. The subject is fasting, takes off his shoes and socks, and wears light-weight clothing. Before the measurement, stop vigorous activities, enter the relevant basic information of the detector, and after preparation, take the biped standing position, place it on the biped electrode, and hold the electrode with both hands. Take 1-2 minutes to measure body fat and body fat rate.

Statistical analysis

Statistical analysis was performed by GraphPad Prism 5 (San Diego, CA). All the quantitative data was presented as the mean $\pm$ standard deviation (SD). The unpaired t test or Mann–Whitney U test was used for comparison between groups according to whether they conform to the normal distribution. Correlations between continuous variables were analyzed by the Pearson correlation test. Multiple linear regression analysis was used to determine the linear correlations between the expression of sOX40 and other variables. P values less than 0.05 was considered as significant difference.

Results

1. sOX40 in T2DM group and control group

A Total of 60 overweight and obese patients with an average age of 32.19 ± 12.19 years were recruited into the study, divided into control group (25 participants) and T2DM group (35 participants). In terms of anthropometric data, BMI of T2DM group is lower than control group ($P < 0.05$). With regard to metabolic parameters and islet function, FBG, HbA_{1c} and HOMA-IR of the T2DM group were significantly higher than the control group ($P < 0.05$); 2hINS, FCP and 2hCP of the T2DM group was significantly lower than the obese group ($P < 0.05$); There were no significant differences in FINS, TC, TG, LDL-C, HDL-C, UA, body fat and body fat rate among two groups ($P > 0.05$). (Table.1)

sOX40 was detectable in both T2DM and control groups. Compared with control group (519.6 ± 14.03), serum levels of OX40 was significantly higher in T2DM group (662.1 ± 15.93). ($p < 0.001$) (Fig. 1)

2. Pearson correlation analysis of HbA_{1c} with other variables

Table 2 showed that the HbA_{1c} was positively correlated to sOX40 and FBG ($r= 0.618$ and 0.629 , $P<0.01$) and negatively correlated to BMI, 2hINS, FCP and 2hCP ($r=-0.380$, -0.524 , -0.306 and -0.485 , $P<0.05$). (Table.2)

Table 2
Pearson correlation analysis of HbA_{1c} with other variables

HbA _{1c}		
Variables	R	P
sOX40(pg/ml)	0.681	<0.01
BMI(kg/m ²)	-0.380	<0.01
FBG(mmol/l)	0.629	<0.01
FINS(pmol/l)	-0.188	0.153
2hINS(pmol/l)	-0.524	<0.01
FCP(ng/ml)	-0.306	0.018
2hCP(ng/ml)	-0.485	<0.01
TC(mmol/l)	-0.083	0.531
TG (mmol/l)	0.035	0.790
LDL-C (mmol/l)	0.066	0.615
HDL-C(mmol/l)	-0.253	0.052
UA(mmol/l)	-0.078	0.554
HbA _{1c} : Glycosylated hemoglobin; sOX40: Solubel OX40; BMI: Body mass index; FPG: Fasting plasma glucose; FINS: Fasting insulin; 2hINS: insulin after 2h meal; FCP: Fasting C-peptide; 2hCP: C-peptide after 2h meal; UA: Uric acid; TC: Total cholesterol; TG: Triglyceride; LDL-C: Low-density lipoprotein cholesterol; HDL-C: High-density lipoprotein cholesterol;		

3. Multivariate linear regression analysis of HbA_{1c} with other variables

Among 60 overweight and obese patients, 35 of them were diagnosed as T2DM. In Multivariate linear regression analysis, increased sOX40($\beta = 0.114$, $P=0.000$) was a significant independent risk factor for hyperglycemia. Meanwhile, increased FBG ($\beta = 0.328$, $P=0.002$) was other independent risk factors for hyperglycemia in overweight and obese patients (Table 3).

Table 3

Multivariate linear regression analysis of HbA1c with other variables in overweight and obese patients

Variable	<i>B</i>	<i>SE</i>	β	<i>T</i>	<i>P</i> value	95% <i>CI</i>
sOX40	0.114	0.027	0.425	4.244	0.000	0.060~0.169
BMI	-0.105	0.061	-0.158	-1.725	0.090	-0.228~0.017
FBG	0.332	0.104	0.328	3.181	0.002	0.123~0.542
2hINS	-0.007	0.005	-0.130	-1.249	0.217	-0.017~0.004
sOX40: Soluble OX40; BMI: Body mass index; FPG: Fasting plasma glucose; 2hINS: insulin after 2h meal						

Discussion

The results of our study demonstrated that soluble OX40 is positively correlated with Glycosylated hemoglobin and is an independent risk factor for hyperglycemia in overweight and obese patients. Therefore, the OX40/OX40L signaling pathway is involved in the progression of insulin resistance mediated and plays an important role in the occurrence of hyperglycemia in overweight and obese patients.

Research on the abnormal expression of costimulatory molecules involved in mediating lymphocyte activation and function in diabetes and its complications is one of the current research hotspots^[11]. OX40/OX40L is the member of the TNFR/TNF super family, which mediates positive stimulation signals. The signal pathway could promote the proliferation and activation of T lymphocyte and inhibit T cell apoptosis, play an extremely important immunomodulatory effect on infiltrating T cells in inflammatory sites, participate in the occurrence and development of inflammation, autoimmunity and other diseases^[12]. Numerous studies have demonstrated that OX40/OX40L signal pathway is important in autoimmune diseases, such as rheumatoid arthritis, Graves' hyperthyroidism and systemic lupus erythematosus^[13–14]. However, the immune mechanism of OX40/OX40L involved in the development of insulin resistance in obesity, type 2 diabetes and other chronic metabolic diseases is largely unknown.

Insulin resistance is a chronic low-grade inflammation, which is a risk factor for hyperglycemia in obesity. It has been reported that the enhancement of OX40/OX40L signal pathway promotes the proliferation, activation of fat-infiltrating T lymphocyte and insulin resistance in obese mice induced by high-fat diet, while, the inflammation immune response of adipose tissue in OX40 knockout mice is reduced^[15–16]. Therefore, the OX40/OX40L signaling pathway plays an important role in the development of insulin resistance in T2DM. Researches in increasing number show co-stimulatory molecules can exist in both membrane and soluble forms. sOX40 is released through proteolytic cleavage of membrane OX40, although any other source cannot be excluded^[17]. The soluble protein factors can participate in blood circulation and play a regulatory role in the immune response like cytokines. They can affect not only the adjacent cells but also the receptor on the surface of the distal cell, so as to participate in the occurrence

and development of the disease^[18]. We performed ELISA analysis and demonstrated that the serum level of OX40 was significantly higher in T2DM group compared with control group in overweight and obese patients. This is a soluble costimulatory molecule, like the molecules on the cell membrane surface, which could mediate immune function and promote the proliferation of T lymphocyte. Although the exact function of sOX40 has not been fully clarified, our study demonstrated that it is an independent risk factor for hyperglycemia in overweight and obese patients. Therefore, we speculate that the sOX40/OX40 signaling pathway plays an important role in the chronic inflammatory response of insulin resistance in obesity patients.

There are some acknowledged limitations in this study. Firstly, since the sample size of this study is small, the conclusions in our findings need to be confirmed in further studies with a larger size. Secondly, we are unable to perform an analysis on the relationship between OX40 expressed on T cells infiltrated in adipose tissue of obesity and serum OX40, which could provide further evidence to characterize the role of OX40 in insulin resistance progression. Finally, the distinct mechanism of the sOX40/OX40L pathway in the pathogenesis of obesity-related diabetes should be further explored.

We will further study the immune mechanism of sOX40/OX40L signaling pathway plays in obesity-induced insulin resistance and chronic tissue inflammation, which will provide us with new treatments for obesity.

Declarations

Authors'contributions

Wang Q and Shi BM developed the study concept and design.

Du X and Zhu Y mainly implemented the study.

Du X, Hu JC, Zhu Y and Lu W registered the study patients and performed physical examination.

Hu JC and Fu NN were responsible for measuring plasma parameters.

Du X and Zhu Y drafted the manuscript.

Du X and Zhu Y performed statistical analyses.

Du X and Hu JC interpreted data and critically revised and completed the manuscript.

All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the institutional review board at the First Affiliated Hospital of Soochow University, and written informed consent was obtained from all participating patients before the initiation

of the study.

Study limitations

Limitations of this study included the small number of study patients. Therefore, further studies are needed to verify the findings in this study.

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Competing interests

There is no conflict of interest.

Availability of data and materials

The datasets analyzed in current study are not publicly available due to relevant ongoing studies, but may be available from the corresponding authors upon reasonable request.

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Figures

Figure 1

Concentration of sOX40 is plotted for each of the 35 cases of T2DM patients and 25 cases of controls. Compared with control group, serum levels of sOX40 were significantly higher in type 2 diabetes patients group ($p < 0.001$).