Correlation Study of Insulin Resistance and Adipocytes Factors in Patients with Type 2 Diabetes

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ABSTRACT

Objective: 30 cases of non-obese patients with type 2 diabetes, 30 cases of obese patients with type 2 diabetes and 30 cases of healthy controls were selected to detect the levels of serum resistin, TNF-α, FFA, fasting blood glucose, fasting insulin, total cholesterol and triglycerides.

Methods: And height, weight and waist circumference, abdominal circumference and blood pressure were measured, then body mass index (BMI) and waist-hip ratio (WHR) were calculated, as well as insulin resistance index (IRI) was calculated using Homa formula.

Results: The results showed that the levels of resistin, TNF-α and FFA in the obese and non-obese diabetic groups were significantly higher than that in the control group, the difference was significant ($p < 0.05$, $p < 0.01$, respectively), the difference of resistin, the differences of TNF-α and FFA levels in the obese diabetic group were significant compared with that in the obese type 2 diabetic group ($p < 0.05$). The blood resistin in the non-obese type 2 diabetic groups had a significantly positive correlation with BMI, FPG and TG, as well as IRI, but no correlation with FINS, TCH, SBP, DBP and WHR.

Conclusions: The resistin, TNF-α and FFA in the non-obese type 2 diabetic group and control groups had no significant correlation with all factors. The results of stepwise regression analysis showed that resistin, TNF-α and FFA were significantly associated with IRI.

Keywords: Adipokines, FFA, insulin resistance, resistin, tumor necrosis factor, Type 2 diabetes

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INTRODUCTION

With the improvement of living standards, diet structure and social aging problem of human beings, the incidence of type 2 diabetes increases every year, and has become a global epidemic. Type 2 diabetes can cause multiple organ damage, the incidence of complications and the mortality serious threat people's health and reduce the people's quality of life. However, the pathogenesis of type 2 diabetes is not very clear yet, therefore, investigating the pathogenesis of type 2 diabetes is a common research for today's diabetes scholars. Recently, American researcher Steppan et al (1) identified a new hormone secreted by fat cells, that is resistin, which plays the role of producing insulin resistance, elevating blood glucose levels, and causing obesity by adipose cell proliferation and reproduction, the synthesis and secretion are affected by regulation of insulin, TNF-α, fatty acids and other cytokines. Resistin may well explain the relationship among insulin resistance, obesity and diabetes, which is a breakthrough in diabetes and obesity research. Meanwhile, the contact between TNF-α and FFA with diabetes and insulin resistance has attracted everyone's attention, and epidemiological and experimental studies have pointed out the TNF-α, FFA's role in insulin resistance. Early prevention and treatment from the point of insulin resistance often prevent or delay the development of type 2 diabetes in a certain extent. Up to now, few studies abroad are about the changes and significance resistin, TNF-α and FFA in insulin resistance, as well as the interactions, but no reports at home.

In this study, the serum concentration changes of resistin, TNF-α, FFA and the interactions with fasting glucose and insulin were individually observed to discuss the main factors affected serum resistin, TNF-α, FFA and the relationship among them and their correlation with insulin resistance.

MATERIALS AND METHODS

All the 90 cases were selected from inpatients and healthy outpatients in our hospital, including 45 males and 45 females, with mean age of (52±13) years old, all subjects had no autoimmune diseases, cancer and chronic infectious diseases, and the tested patients had no acute infectious diseases and ketoacidosis on the day and in nearly two weeks. Three groups

was divided according to glucose (the new DM WHO diagnostic criteria in 1998) and BMI (adult obesity standard in Asia-Pacific region from WHO): non-obese type 2 diabetes (Non-obese DM) group, obese type 2 diabetes (Obese DM) group and normal control group. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Linyi city People's Hospital. Written informed consent was obtained from all participants.

All objects needed to be fasted for 10 hours, and the height, weight, waist and hip circumference were measured by special staff. 5 ml elbow vein morning blood was extracted, sedimented and centrifuged to extract serum for immediate measurement of fasting glucose (FPG), fasting serum insulin (FINS) and cholesterol (TC). Partial serum was stored at -20°C refrigerator to measure fasting serum resistin, TNF-α and FFA concentration. 75 g anhydrous glucose powder was used immediately to patients for extracting fasting blood, 3ml vein blood was picked cubitally two hours later to measure 2-hour postprandial blood glucose. Resistin concentrations were measured by enzyme immunoassay (U.S. BioVendor Laboraory Medicine kit). TNF-α was determined uniformly by enzyme-linked immunosorbent assay (ELISA) in 2 batches. FFA detection was used enzymatic method, and the reagent was provided by English RANDOX companies. INS measurement was performed with the insulin radioimmunoassay kit from China Institute of Atomic Energy using radioimmunoassay method. Blood glucose was determined with glucose oxidase method.

Calculation method for related index

Waist-hip ratio (WHR) = waist/hip

Body mass index (BMI) = weight/height^2 (kg/m)

Insulin resistance was expressed by the insulin resistance of the homeostasis model (Homa Model) Homa IR = (FINS × FPG)/22.5, and the natural logarithm was taken.

All data were inputted into the computer, and analyzed using SPSS10.0 statistical software. The statistical results were expressed as $x \pm s$, the data were tested by normality test or homogeneity of variance test, then the difference among the groups was performed by t test, and correlation coefficients was used to evaluate the correlation among variables, stepwise multiple regression analysis was used to perform multivariate analysis.

RESULTS

The serum resistin, TNF-α and FFA in the normal control group, non-obese diabetic group and obese diabetic group increased in turn, and they were the lowest in the normal control group, followed by non-obese diabetic group, and the highest average was that of the obese diabetic group, there were significant differences between each two groups ($P < 0.05$, Table 1).

Seen from Table 2, the serum resistin level of type 2 diabetic patients were positively correlated with BMI (r=0.40, $P<0.05$), FPG (r=0.37, $P<0.05$), TG (r=0.41, $P<0.05$), IR (r=0.49, $P<0.05$), and no correlated with FINS, TCH, TC, SBP, DBP and WHR. The serum TNF-α level of type 2 diabetic patients were positively correlated with BMI (r=0.406, $P<0.01$), FPG (r=0.274, $P<0.01$), FINS (r=0.428, $P<0.01$), TG (r=0.410, $P<0.01$), FINS (r=0.490, $P<0.01$) TNF-α, but no correlated with WHR, TC, SBP and DB. The serum FFA level of type 2 diabetic patients were significantly correlated positively with BMI (r=0.41, $P<0.01$), FPG (r=0.35, $P<0.01$), FINS (r=0.37, $P<0.01$), TG (r=0.42, $P<0.01$) and FINS (r=0.48, $P<0.01$), but no correlated with WHR, TC, SBP and DB. The resistin, TNF-α and FFA levels of the non-obese type 2 diabetic groups and the control group had no significant correlation with all the factors.

Taking Homa-IR as the dependent variable, and age, BMI, TG, TC, serum resistin and TNF-α, FFA levels as independent variables to perform multiple regression analysis, it was found that Homa-IR was significant correlation with resistin, TNF-α and FFA.

DISCUSSION

Adipose tissues were not only the body's energy storage organ, but also an important endocrine organ. In recent years, the effects of resistin, tumor necrosis factor and free fatty acids secreted by adipose on insulin aroused widespread concern in clinical, several studies showed that these factors were involved in the process of insulin sensitivity damage (2). Resistin was a new peptide signaling molecules found by Steppan in 2001 (1), and the plasma concentrations of the polypeptide was apparent suppressed by the insulin-sensitizing thiazolidinediones (TZDs), it was considered now that resistin is an intermediate medium

between obesity and diabetes. Insulin resistance was a common phenomenon in patients with diabetes; the excessive secreted insulin caused hyperinsulinemia due to the body's decreasing utilization of insulin. Many methods were available to evaluate insulin resistance, and the most common one was calculating Homa IR (IRI) through fasting plasma glucose and fasting insulin levels (3).

In this study, the resistin levels of the normal control group, non-obese diabetic group and obese diabetic group gradually increased, and positively correlated with BMI, FPG, HbA1c and TG, especially IRI reflected the association of resistin concentration with obesity on the one hand, and showed the certain degree of correlation with insulin resistance on the other hand. Domestic scholar Jianliang Zhang studied the serum resistin levels of 71 essential hypertension patients with different glucose tolerance status, and the results showed that resistin levels significantly increased with the aggravation of glucose tolerance impairment, and was positively correlated with blood glucose levels. Recently, Grveleau et al. studied the UCPDTA mice (lack of brown adipose tissue, appeared hyperinsulinemia 12 weeks after birth and diabetes 24 weeks later) and the cardiomyocytes of WT mice (control) and found that resistin can significantly reduce basic intake of insulin-stimulated glucose when glucose existed in myocardial cells medium, while this effect of resistin attenuated in the absence of glucose environment (4, 5).

The possible mechanism may be the binding ability of resistin to insulin receptors on sensitive tissues, then acted to one or several loci of insulin (6) and played the role of anti-insulin-stimulated uptake of glucose. The body would produce insulin resistance when the resistin concentration was too high, then lead to increased blood sugar, suggesting that resistin may impair or inhibit β cell insulin secretion. Further comparison of resistin mRNA levels in different parts of adipose tissues found that the resistin mRNA levels of human abdominal fat pad was 4.18 times higher than that of thigh, which was likely to explain increased risk of the centrally obese type 2 diabetes (7). But there were also reports considered that resistin levels was independent of insulin resistance (8, 9).

TNF-α was a cytokine secreted by activated monocytes, macrophages and adipocytes (10), which involved in the immune protection and immune injury with a dual biological role, and recent researches studied its closely association with insulin resistance

(11). The study found that TNF-α level of patients with diabetes was higher than that of the normal control group, and the concentrations of TNF-α in obese diabetic patients was the highest. Study also found that the TNF-α level of patients with presence of insulin resistance in type 2 diabetes patients was higher than those without insulin resistin (12), which can continually stimulate excessive secretion of pancreatic β cells, eventually lead to depletion of β-cell function (13). Nilsson also believed that TNF-α played an important role in type 2 diabetes insulin resistance (especially in obese patients), TNF-α may be involved in germination of insulin resistance by reducing insulin sensitivity.

The main mechanisms were as follows: 1) reducing expression of glucose transporter 4 (Giut4), 2) inhibiting activity of insulin receptor tyrosine kinase (14), 3) increasing antagonistic hormones, such as insulin ACTH, corticosteroids and adrenaline secretion (15) to induce insulin resistance through antagonistic action of insulin. Meanwhile, the TNF-α levels of type 2 diabetes patients was positively correlated with BMI, FPG, TG and F-INS, indicating that abnormal expression of TNF-α and extreme obesity were closely related to IR state, and resulted in IR in diabetes patients by stimulating growth and inducting volume increasing of fat cells, so the blood glucose control of the obese patients was more difficult compared with that of the non-obese patients.

Recent studies also found that the TNF-α converting enzyme levels in the abdominal fat tissue of the visceral obesity patients was elevated to lead to increased activity of TNF-α and increased insulin resistance, thereby promoted fat synthesis and obesity. In addition, TNF-α was also involved in a number of chronic diabetic complications, such as diabetic nephropathy, retinopathy, atherosclerosis and peripheral neuropathy (16, 17). And this study also found that FFA concentration in diabetic patients was significantly higher than that in non-obese and obese groups. Meanwhile, FFA and blood glucose were also correlated with IRI, suggesting that FFA was also an important factor caused insulin resistance.

TNF-α could not only inhibit gene expression and generation of resistin, but also play a synergistic biological effect for resistin to play the role of insulin resistance. Meanwhile, TNF-α had interaction with resistin. TNF-α was involved in insulin resistance, but TNF-α was a negative regulator of resistin, which could inhibit resistin mRNA expression, suggesting that TNF-α was negative regulatory factor of resistin expression (18, 19), the

process may be associated with PKA and mitogen-activated protein kinase (MAPR) dependent pathway, and other factors led to the elevated resistin levels could not be ruled out (20).

Therefore, in the prevention and treatment of type 2 diabetes, in addition to actively control blood sugar levels, weight losing and active lipid-lowering therapy should be also considered, and thus reduces insulin resistance. Both lose weight or taking insulin sensitizers (thiazolidinediones, TZDs) can stimulate drop of TNF-α levels, correct hyperinsulinemia and reduce insulin resistance (21), and the above effect could also be achieved by using TNF-α inhibitors or TNF-α antibody with the same principle, which has been confirmed by animal experiments (15, 22). Similarly, lowering the resistin levels, counteract the biological activity of resistinin or giving resistin receptor antagonist were the future study direction of drug development.

In summary, more resistin, TNF-α and FFA secreted by adipose tissue of the obese diabetic patients would result in more severe insulin resistance, and the elevated blood sugar levels were also covered by high levels of insulin, so the diabetic patients treated by reducing glucose must pay attention to weight loss at the same time. It should not only prevent high blood sugar but also the occurrence of hyperinsulinemia. Starting from this perspective, the drugs for reducing insulin resistance, such as resistin inhibitors and TNF-α antibody, were developed to reduce insulin resistance, improve hypoglycemic drug efficacy, and finally achieve the purpose of well controlling diabetes.

AUTHORS' NOTE

The authors declare no conflicts of interest.

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Table 1: Serum resistin, TNF-α and FFA measurement results

Group	**Resistin (μg/L)**	**TNF-α (ng/L)**	**FFA (mmol/l)**
Non-obese type 2 diabetes	17.9±4.8*	19.95±6.42*	o.67±0.24
Obese type 2 diabetes	24.5±8.9$^{\Delta\Delta **}$	23.87±6.25$^{\Delta\Delta **}$	1.52±0.87$^{\Delta\Delta *}$
Normal control	13.4±5.2	13.06±4.56	0.46±0.23

Note: Non-obese type 2 diabetes and obese type 2 diabetes versus the normal control group, * P<0.05, ** P<0.01. Obese type 2 diabetes versus non-obese type 2 diabetes, $^{\Delta}$ P<0.05, $^{\Delta\Delta}$ P<0.01.

Table 2: Related analysis of resistin, TNF-α, FFA and other factor

Projects and grouping	**Correlated coefficient**								
	BMI	**WHR**	**FPG**	**FINS**	**TC**	**TG**	**SP**	**DP**	IR
Resistin									
Non-obese diabetes	0.36	0.12	0.25	0.03	0.09	0.09	0.11	0.04	0.36
Obese diabetes	0.40*	0.14	0.37*	0.07	0.11	0.41*	0.15	0.05	0.49**
Normal control	0.17	0.05	0.11	0.02	0.1	0.18	0.06	0.04	0.15
TNF-α									
Non-obese diabetes	0.312	0.129	0.264	0.04	0.11	0.39	0.135	0.04	0.42
Obese diabetes	0.406$^{\Delta}$	0.137	0.274$^{\Delta}$	0.428$^{\Delta}$	0.12	0.41$^{\Delta}$	0.148	0.05	0.49$^{\Delta\Delta}$
Normal control	0.158	0.06	0.11	0.14	0.05	0.07	0.03	0.03	0.12
FFA									
Non-obese diabetes	0.35	0.13	0.24	0.06	0.12	0.29	0.12	0.04	0.41
Obese diabetes	0.41$^{\Delta}$	0.142	0.35Δ	0.37$^{\Delta}$	0.13	0.42$^{\Delta}$	0.14	0.04	0.48$^{\Delta\Delta}$
Normal control	0.16	0.05	0.11	0.10	0.07	0.12	0.04	0.04	0.13

Note: Non-obese type 2 diabetes and obese type 2 diabetes versus the normal control group, * P<0.05, ** P<0.01. Obese type 2 diabetes versus non-obese type 2 diabetes, $^{\Delta}$ P<0.05, $^{\Delta\Delta}$ P<0.01.