Correlation of serum CF6 with blood lipid and glucose in patients with type 2 diabetic retinopathy

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Abstract
This study was to investigate the relationship of serum mitochondrial coupling factor 6 (CF6) with blood lipid and glucose in patients with type 2 diabetic retinopathy. A total of 180 patients with type 2 diabetes enrolled in our hospital from January 2015 to September 2017 were selected as the research objects. They were divided in accordance with fundus fluorescence angiography (FFA) into normal diabetic retinopathy (NDR) group, background diabetic retinopathy (BDR) group as well as proliferative diabetic retinopathy (PDR), with 60 cases in each group, and at the same time, another 60 healthy subjects were selected as normal control (NC) group. Serum CF6, fasting plasma glucose (FPG), 2 h postprandial plasma glucose (2hPG), glycosylated hemoglobin (HbA1c), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), total cholesterol as well as triglyceride were measured in the groups and the relationship of serum CF6 with related indicators was analyzed. The level of serum CF6 in type 2 diabetes group was higher than that in NC group and there was statistically significant difference among the groups with the occurrence and aggravation of retinopathy (P<0.05). Correlation analysis showed that serum CF6 was positively correlated with FPG, 2hPG, HbA1c, and LDL-C of statistical significance (P<0.05) and negatively correlated with HDL-C of statistical significance (P<0.05). The results of multiple step regression analysis showed that HbA1c and LDL-C were independent risk factors for CF6. In conclusion, the serum CF6 of patients with diabetic retinopathy increases with aggravation of the disease, taking a part in the occurrence and development of retinopathy together with the disorder of blood glucose and lipid metabolism.

Keywords
blood glucose, blood lipids, diabetic retinopathy, serum CF6

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Introduction
Type 2 diabetic microvascular complications mainly include such lesions as diabetic retinopathy, diabetic nephropathy, and diabetic neuropathy, seriously affecting patient quality of life.1 The main pathogenesis of diabetic retinopathy has not yet been fully elucidated.2 It is known that coupling factor 6 (CF6) is the only inhibitory factor in human body for endogenous prostacyclin I2 (PGI2) synthesis and mainly distributed in mitochondria as well as vascular endothelial cell membrane. It is a subunit of adenosine triphosphate (ATP) synthase in mitochondria and mainly involved in the conversion of energy substances as well as ATP production. CF6 decreases the release of endogenous arachidonic acid by inhibiting the activity of cytosolic calcium-dependent phospholipase A2,
thereby reducing the production of PGI2.3,4 When the vascular endothelial cells are damaged, the release of CF6 in the blood circulation inhibits the synthesis of PGI2. CF6 is a newly discovered indirect vasoactive factor and its change is related to vascular structure as well as functional lesions.5,6 It is rarely reported in the study of diabetic retinopathy in type 2 diabetic patients. This research is mainly to determine the level of serum CF6 and analyze its correlation with fasting plasma glucose (FPG), blood lipid, glycosylated hemoglobin (HbA1c) as well as blood pressure in diabetic retinopathy patients for exploration of the changes in the concentrations of above related factors so as to further clarify the pathogenesis of the disease.

Data and methods

Clinical data
A total of 180 patients with type 2 diabetes treated in our hospital from January 2015 to September 2017 and with the duration of 1 month to 13 years were selected as the research objects; patients excluded from the study included those with acute complications like diabetic ketoacidosis and hyperosmolar state, inflammatory diseases like infection, injury and rheumatism, blood system diseases and liver disease as well as macrovascular complications like tubular atherosclerotic heart disease, cerebrovascular disease, and lower limb vascular lesion. According to the fundus fluorescein angiography, the patients were divided into normal diabetic retinopathy (NDR) group, background diabetic retinopathy (BDR) group as well as proliferative diabetic retinopathy (PDR), with 60 cases in each group. In the NDR group, there were 32 males and 28 females with an average age of 56.7 ± 3.2 years; in the BDR group, there were 31 males and 29 females with an average age of 56.9 ± 3.4 years; in the PDR group, there were 32 males and 28 females with an average age of 56.7 ± 3.8 years. At the same time, 60 healthy subjects were selected as normal control (NC) group, including 30 males and 30 females with an average age of 56.8 ± 3.6 years. There was no significant difference in gender and age among the four groups (P > 0.05). This study was approved by the Ethics Committee of Eye & Ear Nose Throat Hospital of Fudan University. All patients participated in the study and signed informed consent voluntarily.

Methods

All subjects were fasted for 8 h with the blood pressure measured and recorded in the next morning. Fasting blood (5 m) was collected with a standstill time of about 1 h, the sample was centrifuged at 3000 r/min for 8–10 min followed by separation of serum and cryopreservation at −20°C in refrigerator. (1) The concentration of serum CF6 was determined by radioimmunoassay. (2) The FPG, 2h postprandial blood glucose (2hPG), HbA1c, total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were measured by automatic biochemical analyzer. The patient height, weight, and body mass index (BMI) were calculated.

Statistical methods

SPSS 21 statistical software was used for data analysis, the measurement data were described as mean ± standard deviation (x ± s), among the groups using single-factor analysis of variance, and the pairwise comparison among groups was assessed by least significant difference (LSD)-t test after homogeneity test of variance. Pearson correlation coefficient was calculated to judge the correlation between the two variables. Multiple factor analysis of CF6 was conducted by multiple linear regression method, P < 0.05 suggested there was statistically significant difference.

Results

Comparison of biochemical indexes and serum CF6 in groups

No significant difference of oral hypoglycemic agents was found in NDR, SDR, and PDR groups (Table 1). There was no significant difference in age and BMI in the groups (P > 0.05). Compared with NC group, systolic blood pressure (SBP), diastolic blood pressure (DBP), FPG, 2hPG, HbA1c, and LDL-C were significantly different in NDR group, BDR group, and PDR group (P < 0.05) and with the occurrence and aggravation of retinopathy, FPG, 2hPG, HbA1c, and LDL-C showed a gradually increasing trend.

HDL-C level: NC group > NDR group > BDR group > PDR group, the difference among the groups was statistically significant (P < 0.05).
There was no significant difference in TC level among NDR, BDR, and PDR groups ($P > 0.05$).

TG level: the TG level was decreased in BDR and PDR groups compared with NDR and NC groups with the difference being statistically significant ($P < 0.05$), and there was no statistically significant difference between BDR and PDR groups as well as between NDR group and NC group ($P > 0.05$). The level of CF6: NC group < NDR group < BDR group < PDR group, with the difference among the groups being statistically significant ($P < 0.05$), as shown in Table 2.

### Analysis on the correlation of serum CF6 with other factors

Serum CF6 was positively correlated with FPG, 2hPG, HbA1c as well as LDL-C and negatively correlated with HDL-C, but not correlated with TC and TG, as shown in Table 3.

### Multiple linear regression analysis

Multiple linear regression analysis was conducted with serum CF6 as the dependent variable and BMI, SBP, DBP, FPG, 2hPG, HbA1c, LDL-C, HDL-C, TC as well as TG as independent variables and the results showed that HbA1c and LDL-C are independent risk factors of CF6, as shown in Table 4.

### Discussion

Diabetic retinopathy is the most important manifestation of diabetic microvascular lesions. It is a fundus disease of special changes, one of serious microvascular complications and also a common cause of vision loss in working people. The pathogenesis of diabetic retinopathy is a long-term pathological process and closely related to many factors. Diabetic retinopathy is divided into PDR and non-PDR in which the non-PDR is characterized by retinal edema.

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**Table 1.** Comparison of oral hypoglycemic agents in NDR, SDR, and PDR groups (n=60).

| Group   | Metformin | Sulfonylurea | α-Glucosidase inhibitors | Other oral agents |
|---------|-----------|--------------|--------------------------|------------------|
| NDR     | 38        | 15           | 13                       | 3                |
| SDR     | 36        | 16           | 12                       | 2                |
| PDR     | 39        | 14           | 14                       | 4                |
| F       | 1.296     | 1.986        | 2.112                    | 1.376            |
| P       | 0.134     | 0.101        | 0.093                    | 0.121            |

NDR: normal diabetic retinopathy; PDR: proliferative diabetic retinopathy.

**Table 2.** Comparison of biochemical indexes in groups (n=60).

| Group   | Age (years) | BMI (kg/m²) | SBP (mmHg) | DBP (mmHg) | CF6 (ng/mL) | HbA1c (%) |
|---------|-------------|-------------|------------|------------|-------------|------------|
| NC      | 56.7 ± 3.2  | 23.29 ± 4.36| 123 ± 8    | 75 ± 4     | 63.51 ± 13.47| 4.31 ± 0.42|
| NDR     | 56.9 ± 3.4  | 23.26 ± 4.45| 129 ± 10   | 78 ± 6     | 104.68 ± 17.31| 7.25 ± 1.67|
| SDR     | 56.7 ± 3.8  | 23.17 ± 4.26| 131 ± 11   | 80 ± 7     | 183.24 ± 19.79| 9.32 ± 2.19|
| PDR     | 56.8 ± 3.6  | 23.12 ± 4.21| 145 ± 14   | 84 ± 8     | 301.44 ± 23.21| 11.02 ± 2.84|
| F       | 1.478       | 3.692       | 24.095     | 28.177     | 45.093      | 47.164     |
| P       | 0.124       | 0.076       | 0.000      | 0.002      | 0.000       | 0.000      |

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; NC: normal control; NDR: normal diabetic retinopathy; PDR: proliferative diabetic retinopathy; FPG: fasting plasma glucose; 2hPG: 2 h postprandial plasma glucose; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; TC: total cholesterol; TG: triglyceride.
resulting from abnormal retinal permeability as well as retinal non-perfusion and ischemia caused by closing of capillary vessel.\textsuperscript{9,10} Diabetic retinopathy is a potential cause of blindness in diabetic patients. The diagnosis and treatment focus on vascular malformations occurring in the later stage of the disease. Related studies have shown that\textsuperscript{11} diabetic patients mainly experience changes in eye tissue, nerve, and vascular microcirculation due to the abnormalities of insulin and cell metabolism, ending up in suffering from the damages of eye nutrition and visual function. The change of blood components in diabetic patients will lead to vascular endothelial cell dysfunction and then destroy blood–retinal barrier. The microvascular lesions in diabetic patients, mainly occurring in retina and kidney, are the main causes of blindness, renal failure, and death. A study has suggested that\textsuperscript{12} almost 100% of those with more than 20-year duration of type 2 diabetes will suffer from DR. Therefore, to carry out series of researches on the pathogenesis, diagnose and treatment of DR is of very important significance for blindness prevention and treatment as well as improvement of quality of life in patients.

CF6 not only exists on not only the surface but also the plasma membrane of vascular endothelial cells, and the injured endothelial cells may release CF6 into the blood circulation.\textsuperscript{3} DR has been shown in present studies to be associated with systemic vascular endothelial cell damage caused by hyperglycemia in patients with type 2 diabetes mellitus.\textsuperscript{13} The results of this research showed that serum CF6 in type 2 diabetes group was higher than that in NC group, and it was gradually increased with the occurrence and aggravation of retinopathy, positively correlated with FPG, 2hPG, HbA1c, and LDL-C (\(P < 0.05\)). Multiple stepwise regression analysis showed that HbA1c and LDL-C were independent influencing factors of serum CF6 level, indicating that CF6 was involved in the occurrence and development of retinopathy with glucose metabolism and lipid metabolism disorders. It is also suggested that type 2 diabetic retinopathy is related to systemic vascular endothelial cell injury with the condition significantly paralleled with the severity of DR. The increase of CF6 level may result from the long-term hyperglycemia-induced vascular endothelial cell damages. In addition, the type 2 diabetic patients usually suffer from disorders of lipid metabolism, which accelerates irreversible and progressive lesions of the blood vessels. We believe that the elevated serum concentration with the release of CF6 may serve as a measure of vascular structural and functional lesions and the measurement of its changes may help to explain the pathogenesis of DR.\textsuperscript{14}

To sum up, through this experiment further elucidates the pathogenesis of diabetic retinopathy by studying the changes of CF6 level and its correlation with blood lipids as well as blood glucose in patients with type 2 diabetic retinopathy, providing a theoretical basis for seeking indexes for the early diagnosis of diabetic retinopathy and working out effective intervention measures.

**Declaration of conflicting interests**

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**Table 3. Analysis on the correlation of serum CF6 with other factors.**

| Factor | \(r\) | \(P\) |
|--------|-------|-------|
| SBP    | 0.284 | 0.123 |
| DBP    | 0.172 | 0.161 |
| FPG    | 0.285 | 0.026 |
| 2hPG   | 0.394 | 0.009 |
| HbA1c  | 0.361 | 0.012 |
| LDL-C  | 0.435 | 0.002 |
| HDL-C  | -0.313| 0.003 |
| TC     | 0.195 | 0.144 |
| TG     | 0.176 | 0.238 |

CF6: coupling factor 6; SBP: systolic blood pressure; DBP: diastolic blood pressure; FPG: fasting plasma glucose; 2hPG: 2 h postprandial plasma glucose; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; TC: total cholesterol; TG: triglyceride.

**Table 4. The results of analysis with CF6 as a dependent variable.**

| Variable | \(b\) | \(S_b\) | \(\beta\) | \(t\) | \(P\) |
|----------|-------|---------|--------|------|------|
| HbA1c    | 0.013 | 0.006   | 0.008  | 2.795| 0.014|
| LDL-C    | 0.082 | 0.041   | 0.045  | 2.343| 0.032|
| Constant | 0.364 | 0.146   | –      | 2.907| 0.012|

CF6: coupling factor 6; LDL-C: low-density lipoprotein cholesterol
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