·Clinical Research·

Relationship between modified homeostasis model assessment/correlative serum factors and diabetic retinopathy among type 2 diabetics with insulin therapy in Guangzhou, China

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Abstract

• AIM: To explore the related risk factors for diabetic retinopathy (DR) in type 2 diabetes with insulin therapy.

• METHODS: We studied the relationships among blood glucose, serum C-peptide, plasma insulin, beta-cell function and the development of DR. Beta-cell function was assessed by a modified homeostasis model assessment (modified HOMA) which was gained by using C-peptide to replace insulin in the homeostasis model assessment (HOMA) of beta-cell function. We also studied the relationships between modified HOMA index and serum C-peptide response to 100 g tasteless steamed bread to determine the accuracy of modified HOMA.

• RESULTS: Our study group consisted of 170 type 2 diabetic inpatients with DR (age: 58.35±13.87y, mean±SD) and 205 type 2 diabetic inpatients with no DR (NDR) (age: 65.52±11.59y). DR patients had higher age, longer diabetic duration, higher hypertension grade, higher postprandial plasma glucose, higher fluctuation level of plasma glucose, lower body mass index (BMI), lower postprandial serum insulin and C-peptide, lower fluctuation level of serum insulin and C-peptide(P<0.05). In our logistic regression model, duration of diabetes, hypertension grade, fasting plasma insulin and glycosylated hemoglobin (HbA1C) were significantly associated with the presence of DR after adjustment for confounding factors (P<0.05).

• CONCLUSION: Our results suggested although modified HOMA showed significant correlation to the occurrence of DR on Spearman's rank-correlation analysis, logistic regression showed no significant association between these two variables after adjustment for relevant confounding factors (such as age, sex, duration of diabetes, BMI, hypertension grade, HbA1C, plasma insulin). Duration of diabetes, hypertension grade, fasting plasma insulin and HbA1C were independently associated with the development of DR in Chinese type 2 diabetics.

• **KEYWORDS:** modified homeostasis model assessment; diabetic retinopathy; insulin

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INTRODUCTION

As the worldwide prevalence of diabetes increases year by year, its incidence is estimated to reach 5.4%, with an estimated 299.974 million global diabetic population by the year 2025 [1]. With this increasing incidence, diabetic retinopathy (DR) becomes one of the leading causes of adult blindness. So, the efforts to prevent DR should be aimed at the risk factors resulting in the genesis of DR among type 2 diabetic. At present, plenty of research discovered many risk factors of DR, such as diabetic duration, long-term poor glycemic control, decreased islet β cell function, and so on[2-5]. We constantly use homeostasis model assessment (HOMA) to evaluate islet β cell function, but insulin therapy interferes with the endogenous insulin secretion in type 2 diabetics, so HOMA is inapplicable for insulin-treated patients [6,7]. In China, Li *et al* [8] found a modified homeostasis model assessment (modified HOMA) using C-peptide to replace insulin in HOMA to evaluate islet β cell function among diabetics with insulin therapy. To the best of our knowledge, there are no previously published studies to investigate the accuracy of this new index. On the other hand, few studies analyzed the practicality of modified HOMA in diabetics

with exogenous insulin therapy. The purpose of this research is to determine whether the modified HOMA is related to residual β cell function assessed by serum C-peptide fluctuation response to 100 g tasteless steamed bread and the occurrence of DR among type 2 diabetics[9].

SUBJECTS AND METHODS

Subjects We screened 375 in-patients with type 2 diabetes, who were admitted to the Third Affiliated Hospital of Guangzhou Medical College in China from January 2005 to May 2010 with either poor glycemic control or for initiation of therapy in newly diagnosed Diabetes. In this study, we obtained informed consent of all patients and ethics committee, we also complied with declaration of Helsinki. Diagnosis of diabetes mellitus was based on the World Health Organization definition, subjects with fasting plasma glucose (FPG) level ≥7.0 mmol/L or 2-h post-load value ≥11.1 mmol/L were defined as diabetic patients [10]. The inclusion criteria of our study were as follow: 1) the subjects were in-patients with type 2 diabetic who didn't have other conditions that may influence the value of plasma glucose, insulin, C-peptide, such as pregnant female, patients treated with any postmenopausal hormone replacement therapy; 2) The patient has received treatment with insulin. Among these subjects, 165 men and 210 women were included in the study, their age and diabetes duration (mean±1SD) were 61.60±13.36 and 6.00±6.37y respectively.

Assessment of Diabetic Retinopathy Fundus photography was done (using Kowa VX-10a Fundus Camera) and fundus records recorded by ophthalmologists using direct and indirect ophthalmoscopy after pupil dilatation with 0.5% tropicamide, were reviewed to evaluate patients' retinae. The diagnosis of DR was confirmed with any of the following: microaneurysms, intraretinal hemorrhages, definite venous beading, prominent intraretinal microvascular abnormalities, neovascularization, vitreous/preretinal hemorrhage [11]. Diabetic subjects were divided into DR group and no DR (NDR) group according to the worse eye.

Measurement of Biochemical Markers Height and weight were measured by the same nurse. All subjects wore light clothing but not shoes, body mass index (BMI) was calculated as weight in kilograms divided by the square of the height in meters. Hypertension was divided into 3 grades: the first grade was defined as a systolic/diastolic blood pressure of 140-159/90-99 mm Hg, the second grade was 160-179/100-109 mm Hg, the third grade was ≥180 /≥110 mm Hg. After an overnight fast, venous blood samples were collected from the patients before and 1, 2, 3h after the oral ingestion of 100 g tasteless steamed bread. Fasting glycosylated hemoglobin (HbA1C) was measured by chromatography, fasting and postprandial plasma glucose were measured by enzyme electrodes, fasting and postprandial plasma insulin and C-peptide were measured by chemiluminescence. By subtracting the fasting plasma glucose (FPG) value from 1st hour, 2nd h and 3rd h postprandial plasma glucose (1st PPG, 2nd PPG, 3rdPPG) values, we got the value of the △1st, △2nd, and △3rd plasma glucose (△1stPG, △2ndPG, △3rdPG). Using the same method from the value of fasting plasma insulin and C-peptide (FPI, FCP) and 1st h, 2nd h, 3rd h postprandial plasma insulin and C-peptide (1stPPI, 2ndPPI, 3rdPPI, 1st CP, 2nd CP, 3rd CP), we could get the value of △1st, △2nd, △3rd plasma insulin or C-peptide (△1stPI, △2nd PI, △3rdPI, △1stCP, △2ndCP, △3rdCP).

Evaluation of Beta-cell Function In this study, insulin therapy was administered in all subjects, however, the HOMA of beta-cell function was not applicable. So we used modified HOMA to evaluate islet beta-cell function. This new formula was described as follows: Beta-cell function (modified HOMA beta-cell)=0.27×FCP/(FPG-3.5).

Statistical Analysis The data was expressed as mean±SD and all the analysis was performed by SPSS 16.0, the independent *t* test method was used to evaluate the differences of the means for continuous variables between subjects with DR and subjects without DR. In this study, excluding 1stPPG, 2nd PPG, 3rdPPG, △1stPG, △2ndPG and △3rdPG, other variables were not normally distributed, so the Mann-Whitney *U* test was used. The relationship between Beta-cell function and the development of DR was evaluated by using Spearman's rank correlation coefficients. This correlation test was also used to assess the relationship between Beta-cell function and △1stCP, △2ndCP, △3rdCP. Binary logistic regression was used to investigate the risk factors of the development of DR in type 2 diabetics.

RESULTS

As shown in Table 1, age, duration of diabetes, hypertension grade, 2ndPPG, 3rdPPG, △2ndPG and △3rdPG level were significantly higher in DR group than in the NDR group. On the other hand, BMI, HbA1C, 1stPPI, △1stPI and △2ndPI value, FCP level, 1stCP, 2ndCP, 3rdCP, △1stCP, △2ndCP and △3rdCP level were significantly higher in NDR group than in DR group. There were significant differences between the groups with respect to sex and beta-cell function (calculated by modified HOMA beta-cell). FPG, 1stPPG and △1stPG, FPI, 3rdPPI and 2ndPPI, △3rdPI value were similar between the two groups.

In the logistic regression model showed in Table 2, duration of diabetes, hypertension grade, FPI and HbA1C were significantly associated with the presence of DR after adjustment for age, sex, BMI, blood glucose, serum

Table 1 Clinical characteristics of type 2 diabetic patients with and without diabetic retinopathy $\bar{x} \pm s$

Parameters	Group 1 (without diabetic retinopathy, NDR group)	Group 2 (with diabetic retinopathy, DR group)	*P*
n	205	170	-
Male/female	109/96	56/114	0.000
Age (a)	58.35±13.87	65.52±11.59	0.000
Duration of diabetes (a)	3.12±3.76	9.49±7.11	0.000
BMI (kg/m^2)	24.49±3.31	23.49±3.02	0.008
Hypertension grade	1.01±1.20	1.85±1.19	0.000
HbA1C(%)	9.99±2.55	9.33±2.23	0.018
FPG(mmol/L)	7.35±2.04	7.46±2.11	NS
1stPPG(mmol/L)	16.63±3.37	16.50±3.47	NS
2ndPPG(mmol/L)	18.99±4.19	20.12±3.99	0.008
3rdPPG(mmol/L)	16.73±4.69	19.11±4.55	0.000
△1stPG(mmol/L)	9.28±2.71	9.05±3.05	NS
△2ndPG(mmol/L)	11.64±3.50	12.67±3.77	0.006
△3rdPG(mmol/L)	9.38±3.95	11.65±4.34	0.000
FPI(mU/L)	8.64±4.77	9.80±6.17	NS
1stPPI (mU/L)	28.92±25.27	23.91±19.51	0.026
2ndPPI(mU/L)	38.24±36.76	31.84±27.87	0.060
3rdPPI(mU/L)	29.62±28.13	27.51±26.64	NS
△1stPI(mU/L)	20.27±22.95	14.11±17.17	0.000
△2ndPI(mU/L)	29.59±34.60	22.04±25.49	0.004
△3rdPI(mU/L))	20.98±25.38	17.71±24.33	NS
FCP(pmmol/L)	565.45±440.45	512.39±442.73	0.016
1stCP(pmmol/L)	1427.58±913.21	1138.60±814.65	0.001
2ndCP(pmmol/L)	2215.39±1234.79	1795.93±1167.77	0.000
3rdCP(pmmol/L)	2243.28±1269.03	1984.24±1250.59	0.018
△1stCP(pmmol/L)	862.13±680.80	626.21±569.28	0.000
△2ndCP(pmmol/L)	1649.94±1049.28	1283.54±949.17	0.000
△3rdCP(pmmol/L)	1677.84±1092.81	1471.85±1044.38	0.032
Modified HOMA beta-cell	45.05±64.31	59.83±203.13	0.042

NS: Not significant ($P>0.05$).

Table 2 Univariate logistic regression analysis with diabetic retinopathy as the dependent variable in type 2 diabetes

Parameters	β /odds ratio	95% confidence interval	*P*
Duration of diabetes (a)	0.168	1.111-1.259	0.000
Hypertension grade	0.390	1.144-1.909	0.003
HbA1C (%)	-0.244	0.680-0.904	0.001
FPI (mU/L)	0.080	1.012-1.159	0.021
Modified HOMA β -cell	0.001	0.996-1.005	NS

NS: Not significant ($P>0.05$).

C-peptide). No significant association was presented between modified HOMA beta-cell index and the occurrence of DR in type 2 diabetes by adjustment for relevant confounding factors (such as age, sex, duration of diabetes, BMI, hypertension grade, HbA1C, plasma insulin).

DISCUSSION

So far, many studies explored the relationship between the islet β-cell function and development of DR in type 2 diabetic, most of which used the HOMA formula to evaluate islet β-cell secretion level. This formula only applies to patients with no insulin therapy. Nonetheless, a large proportion of type 2 diabetes with DR needed insulin injections to control blood sugar, so the applicable scope of the HOMA formula was narrow [5,12]. In our study, we used modified HOMA formula to evaluate the islet beta-cell function. The results showed there was positive correlation between modified HOMA index and the postprandial C-peptide fluctuations level, and the fluctuation level of plasma C-peptide could assess the residual beta-cell function[9]. So we believe that the modified HOMA formula was

practical on evaluation of the beta cell function in type 2 diabetics with insulin therapy. Although modified HOMA showed weekly significant correlation to the occurrence of DR on Spearman's rank-correlation analysis, logistic regression showed no significant association between these two variables after adjustment for relevant confounding factors (such as age, sex, duration of diabetes, BMI, hypertension grade, HbA1C, plasma insulin).

Many previous studies summarized that long duration of diabetes played an important role in the occurrence of DR, and our study also supported this conclusion[10,13]. In the past, investigators usually detected fasting plasma glucose to judge the control condition of plasma glucose, but the accuracy was affected by many factors, such as diet, drug, the time of blood collection. So many studies focused on postprandial plasma glucose [14-16]. However, this kind of studies mostly analyzed the relationship between macrovascular complication and postprandial blood glucose and a few of researches focused on diabetic retinopathy, meanwhile, the results conflicted with each other [17,18]. In our study, the 2nd and 3rd h postprandial plasma glucose level were significantly different between DR and NDR groups, and fasting plasma glucose was similar between the two groups. Beside this, there was significant relationship between the development of DR and 2nd, 3rd h postprandial plasma glucose, while the development of DR and fasting plasma glucose was not related. In our opinion, compared to fasting glucose, it was more important to detect and control the level of postprandial plasma glucose. As for no difference in fasting plasma between two groups (DR and NDR group), we believed that the occurrence of DR needed a long-term bad glucose control, but fasting plasma only represented one night glucose control. Recent clinical studies also confirmed that high fluctuant plasma glucose significantly increased the incidence rate of microvascular diseases and cardiovascular complications in diabetic patients, and the more dangerous fluctuation existed in blood sugar, the greater risk of chronic complications would occur in diabetes[19,20]. High blood sugar fluctuation stimulated the generation of reactive oxygen species and free radicals, increased oxidative stress by protein kinase C, aroused abnormal vascular reactivity and hypercoagulability, and raised endothelial inflammation. At last, it promoted apoptosis of endothelial cells, and accelerated the development of diabetic complications[21,22]. In addition, the fluctuations of blood glucose closely correlated with β-cell function [23]. The results of our study showed that there were significant relationship between △2nd, △3rd h postprandial plasma glucose and the occurrence of DR. It also suggested that the higher fluctuations of blood glucose had some correlation with the development of DR statistically. So we should pay attention to the coordination between the control of fasting plasma glucose and postprandial glucose. If we only emphasize fasting glucose level up to the standard, and overlook the control of postprandial glucose, it will lead to the increasing of blood glucose fluctuation level. At the same time, if the fasting blood sugar level drops too low, even though the postprandial blood glucose level is in the normal extent, blood sugar fluctuations will also increase. In the end, the development of DR will be unavoidable. In conclusion, clinicians should control the level of fasting plasma glucose and postprandial blood glucose within the normal range simultaneously, meanwhile, it is quite important to avoid the incidence of hypoglycemia.

In recent years, a great deal of studies concerned the advantages and disadvantages of the insulin therapy in diabetes. Long time ago, insulin therapy was known as the all-right method in control of plasma glucose level and decreased occurrence rate of some diabetic complications. However with the indepth research, the positive role of insulin had also been suspected. European prospective meta-analysis studies showed that after controlled possible confounding factors, high fasting plasma insulin level increased the cardiovascular mortality by 1.5 times for men, 2.7 times for women [24]. In other studies considered that external insulin promoted the development of DR, the reason was insulin therapy stimulated the expression of insulin-like growth factor-I receptor, insulin-like growth factor-I receptor could upregulate the level of vascular endothelial growth factor (VEGF) levels, and VEGF promoted neovascularization to result in aggravating lesions of the DR[25]. Moreover, insulin increased the quantity and adhesion of leukocytes in the microcirculation of DR, aggravated ischemia and hypoxia in microcirculation, affected permeability of blood-retinal barrier, followed by a series of pathological changes, such as bleeding, oozing, neovascularization [26,27]. In our logistic regression model, fasting plasma insulin was the risk factor for DR, the odd ratio was 0.080 (>0), so we believed that high fasting insulin level would lead to the development of DR. In our study, postprandial insulin level was negatively correlated with the occurrence of DR, which meant that high postprandial insulin level slowed the development of DR. Regarding the reason for the above results, we believed fasting insulin level was influenced by external insulin therapy, postprandial insulin levels was less affected by external insulin, and the worse condition of diabetic needed the more external insulin therapy. So our study showed

higher fasting insulin level would lead to the development of DR. We should also pay attention to the fluctuating level of postprandial insulin, because the correlation coefficients between the fluctuating level of postprandial insulin and the occurrence of DR was higher than that between postprandial insulin level and the development of DR, besides we believed the fluctuating level of postprandial insulin was better representative for physical insulin secretory function than postprandial insulin level.

Several studies showed C-peptide had clinical applicability, and it could mitigate or reverse microvascular complications of diabetes such as diabetic nephropathy, diabetic neuropathy, and DR. Treatment with C-peptide could improve the glomerular filtration rate and reduce protein leakage [28,29]. C-peptide had the effect in improving the velocity of motor nerve conduction and increasing sodium-potassium-ATP enzyme (Na +-K +-ATP enzyme) activity of sciatic nerve [30]. Therapy combined with insulin and C-peptide had less fluorescein leakage by blood-retinal barrier than therapy with insulin alone in type 1 diabetes[31]. C-peptide therapy could increase retinal blood flow and reduce retinal vascular permeability [32]. The existed researches between C-peptide and diabetes mostly concentrated on patients with type 1 diabetes, our study had its novelty because of the subjects with type 2 diabetes. The results of our study showed the level of fasting C-peptide, postprandial C-peptide and fluctuations of postprandial C-peptide were all statistical different between DR and NDR groups, and the correlation coefficients between variables about C-peptide and the occurrence of DR were higher than those between variables about insulin and the occurrence of DR on the corresponding time point. In our opinion, external factors affected the level of plasma C-peptide lightly, so we believed the level of C-peptide was better representative for Physical condition of type 2 diabetes than plasma glucose and insulin level.

However, there were some limitations in our study. First, the subjects were all type 2 diabetic inpatients, so the selection bias was inevitable, this may explain why HbA1C was negatively associated with the development of DR. Second, our study was retrospective, its results needed to be proved with prospective study. We will continue to do follow-up study to make up these defects.

In conclusion, the modified HOMA formula was practical on evaluation of the beta cell function in type 2 diabetics with insulin therapy. After adjustment for confounders, duration of diabetes, hypertension grade, fasting plasma insulin were independently associated with the development of DR in Chinese type 2 diabetics. In addition, postprandial insulin, fasting and postprandial C-peptide the fluctuation level of postprandial plasma glucose, insulin and C-peptide were useful for the diagnosis of DR.

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