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Glucagon secretion is increased in patients with Type 2 diabetic nephropathy

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ABSTRACT

Aims: Currently little is known about the relationship between renal function, albuminuria and glucagon; we analyzed the secretion of glucagon (GLA) and C-peptide in Type 2 diabetic patients with different degrees of nephropathy.
Methods: 357 patients with Type 2 diabetes including 119 cases without nephropathy and 238 cases with nephropathy were divided into four groups according to the stages of diabetic nephropathy. Patients with diabetic nephropathy were further classified according to the level of estimated glomerular filtration rate (eGFR). OGTT and insulin, C-peptide, glucagon releasing tests were performed in all patients. Characteristics of glucagon and C-peptide secretion in different groups were compared. Glucagon/glucose ratio (GLA/GLU) and glucagon/insulin ratio (GLA/INS) were used to represent the inhibition of glucose or insulin on glucagon secretion, respectively.
Results: With the progress of diabetic nephropathy, glucagon level increased significantly; the glucagon peak after glucose load delayed from 60 min to 120 min, whereas C-peptide level decreased significantly. Related factors analysis suggested that glucagon was independently correlated with eGFR. Further analysis showed that glucagon level was higher in group with eGFR < 60 ml/min compared with that in group with eGFR ≥ 60 ml/min. In addition, both GLA/INS and GLA/GLU were higher in group with eGFR < 60 ml/min compared with those in group with eGFR ≥ 60 ml/min.
Conclusions: Patients with Type 2 diabetic nephropathy have worsened islet alpha and beta cell function. Therefore medications based on the regulation of glucagon secretion may improve glycemic control and also be beneficial for delaying the progress of diabetic nephropathy.

1. Introduction

Diabetic nephropathy (DN) is one of the most important microvascular complications of Type 2 diabetes, and is an important cause of ESRD. The guideline of American Diabetes Association suggests that (Association, 2013), an annual test should be performed to assess urine albumin excretion in Type 1 diabetic patients with diabetic duration of ≥5 years and in all Type 2 diabetic patients starting at diagnosis. Serum creatinine should be measured at least annually in all adults with diabetes regardless of the degree of urine albumin excretion to estimate GFR and stage the level of chronic kidney disease (CKD), if present. Researches have shown that many hormones vary in patients with different levels of eGFR. Research of Jianbin Su showed that in patients with pre-diabetes, fasting insulin is positively related to eGFR (Jianbin, Xueqin, Jinfeng, et al., 2010). In another study, free triiodothyronine is proved to have a downward trend with the decline of eGFR, even if the thyroid function is in the normal range, while elevated TSH is proved to be negatively correlated with renal function (Asvold, Bjøro, & Vatten, 2011). Diabetes mellitus is a dual hormone disease, and its pathogenesis is not only related to the secretion and effect of islet beta cell (Gong & Muzumdar, 2012), but also closely associated with islet alpha cell function. The classical view of diabetes claimed that the absolute lack of insulin in T1DM and the relative lack of insulin in T2DM are the main pathogenetic factors of diabetes in human. But in the early 1970's, Unger (Unger, Aguilar-Parada, Müller, et al., 1970) and his colleagues found that alpha cell function increased in diabetes mellitus and proposed that the dysfunction of glucagon plays a significant role in the diabetic syndrome. Glucagon, which is secreted

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by islet alpha-cell, is a 29 amino acid peptide hormone (Christensen, Bagger, Vilsbøll, et al., 2011). Glucagon plays an essential role in the regulation of hepatic glucose production; fasting and postprandial plasma glucagon concentrations are increased in patients with Type 2 diabetes (T2DM), which contribute to the hyperglycaemia (Holst, Christensen, Lund, et al., 2011). In Type 1 diabetes, glucagon might also contribute to glycemic instability (Bessho, Murase-Mishiba, Tsutsumi, et al., 2013). In vivo (Jiang & Zhang, 2003) and in vitro experiments have showed that glucagon can regulate glucose metabolism through promoting liver glycogen decomposition, enhancing sugar dysplasia, inhibiting glycogen synthesis and glycolysis. In non-diabetic population, glucagon rises in 30 minutes after meal, then gradually decreases and is slightly below the fasting level at 180 minutes; compared with fasting, the level of GLA/INS in 30 minutes after meal decreases rapidly, reaching at the lowest level at 60 minutes after meal and then rises to fasting level in 180 minutes (Kahn et al., 2007). Researches have shown that, in diabetic population, the level of fasting glucagon is not different from that in non-diabetic patients, but after glucose load the glucagon level is much higher than that in non-diabetic patients (Unger et al., 1970). Moreover, in patients with impaired glucose tolerance or Type 2 diabetes, with the elevation of insulin level, glucagon also increases significantly (Unger, 1978). But now much more attention have been paid to the use of glucagon in glycemic control and energy metabolism, along with its clinical application with hypoglycemia (Gylfe & Gilon, 2014; Li & Zhuo, 2007), but less is focused on the change of glucagon levels in patients with Type 2 diabetic nephropathy. The relationship between renal function and glucagon is still unclear. In this study we analyzed the characteristics of glucagon and C-peptide secretion after glucose load in patients with Type 2 diabetic nephropathy.

2. Subjects, Materials and Methods

2.1. Subjects

We studied 357 cases of inpatients with Type 2 diabetes (1999 WHO Diagnostic Criteria), including 218 male and 139 female with the average age of (56.0 ± 9.8) years and the average diabetes duration was(8.9 ± 6.7)years. Patients with acute cardiovascular and cerebrovascular events, infection, stress state, other endocrine metabolic diseases, a recent ketoacidosis or hyperosmotic nonketonic coma were excluded. Based on the diagnostic criteria of diabetic nephropathy (The guideline of American Diabetes Association-2014), patients with diabetes were classified into four groups according to 24hMAU, 24hUTP and eGFR: normal group (MAU < 30 mg/24 h and eGFR < 125 ml · min^{-1} · 1.73^{-2}, NDN group), early diabetic nephropathy group (30 mg/24 h ≤ MAU < 300 mg/24 h and UTP < 0.5 g/24 h, DN3 group), clinical diabetic nephropathy group (MAU > 300 mg/24 h or UTP > 0.5 g/24 h,with eGFR ≥ 90 ml•min^{-1}•1.73^{-2}, DN4 group) and renal failure group (MAU > 300 mg/24 h or UTP > 0.5 g/24 h,with eGFR < 90 ml•min^{-1}•1.73^{-2}, DN5 group). And then according to the recommendation of American Kidney Foundation K/DOQI panel for stages of chronic kidney disease (CKD), patients with diabetic nephropathy (group DN3, DN4 and DN5) were further divided into two groups according to levels of eGFR since eGFR is a significantly independent related factor of fasting glucagon: GFR1 group (eGFR ≥ 60 ml · min^{-1} · 1.73^{-2}), and GFR2 group(eGFR < 60 ml · min^{-1} · 1.73^{-2}). This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Tianjin Medical University, China.

2.2. Research methods

The clinical data of patients were analyzed, including general data, laboratory examination, etc. Blood lipids and other biochemical indicators were assayed by automatic biochemistry analyzer (Germany Bayer). Glycosylated hemoglobin (HbA1c) was assayed by HPLC.

Oral glucose tolerance test (OGTT) and insulin, C-peptide, glucagon releasing test: after fasting for more than 10 hours, all patients orally took 75 g anhydrous glucose powder dissolved in 300 ml water; and then the venous blood were taken to assay blood glucose (hexokinase method), insulin (electrochemical luminescence method), C-peptide (RIA) and glucagon (RIA) at 0, 30, 60, 120 and 180 min.

Glucagon/insulin ratio (GLA/INS) and glucagon/glucose ratio (GLA/GLU) were used respectively to evaluate the inhibition of insulin or glucose on glucagon secretion. The simplified MDRD (Modification of Diet in Renal Disease) formula was used to calculate eGFR: eGFR = 186 × (serum creatinine/88.4)$^{-1.154}$ × (age)$^{-0.203}$(female × 0.742).

2.3. Statistical Analysis

SPSS 17.0 software was used for the analysis. Normal distribution data were expressed as ($\bar{x} \pm s$). Non-normal distribution data were transformed by natural logarithm before analysis. Single factor analysis of variance (ANOVA) (LSD or Dunnett T3) test was used for comparison among groups; independent sample T test was used for comparison between GFR1 and GFR2 group. Pearson correlation analysis and multiple stepwise regression analysis were performed to analyze the association between glucagon and other indicators, $P < 0.05$ was considered statistically significant.

3. Results

3.1. Comparison of clinical characteristics among groups

Gender, BMI, FPG, FINS and HbA1c among groups were comparable. With the progress of diabetic nephropathy, age and duration increased significantly ($P < 0.05$). The mean arterial pressure (MAP) and total cholesterol (TC) in three diabetic nephropathy groups were higher than those in NDN group ($P < 0.05$). The plasma albumin (ALB) in DN5 was lower whereas the uric acid and fibrinogen in DN5 were higher than those in other groups ($P < 0.05$). The differences of triacylglycerol (TG) and high density lipoprotein cholesterol (HDL-C) among groups were not statistically different ($P > 0.05$) (Table 1).

3.2. Glucagon levels in different groups

With the progress of diabetic nephropathy, there was an increasing trend in the level of glucagon. Glucagon at different time after glucose load in patients with diabetic nephropathy were higher than that in patients without diabetic nephropathy ($P < 0.05$).

In non-diabetic nephropathy group, the peak of glucagon level was (139.28 ± 33.21) pg/ml, which occurred at 60 min after glucose load. In DN3, DN4 and DN5 group, the peaks of glucagon level were (167.77 ± 70.25) pg/ml, (184.82 ± 79.16) pg/ml and (184.97 ± 57.55) pg/ml, respectively, which were much higher than that in non-diabetic nephropathy group and the peaks of group DN3, DN4 and DN5 were delayed to 120 min (Fig. 1).

3.3. C-peptide levels in different groups

With the progress of diabetic nephropathy, the level of C-peptide showed a decreasing trend. C-peptide at different time after glucose load in patients with diabetic nephropathy were lower than that in patients without diabetic nephropathy ($P < 0.05$) (Fig. 2).

3.4. Glucagon /C-peptide ratios in different groups

With the progress of diabetic nephropathy, the level of glucagon/C-peptide ratio showed an increasing trend; glucagon/C-peptide ratio in patients with diabetic nephropathy were higher than that in non-diabetes group ($P < 0.05$). The levels of fasting glucagon/