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Original Article

The investigation of glucose metabolism and insulin secretion in subjects of chronic hepatitis B with cirrhosis

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Abstract: Aims: To investigate the situations of abnormal glucose metabolism and dysfunction of pancreatic islet beta cells in subjects of chronic hepatitis B (CHB) with cirrhosis. Methods: 106 hepatitis B virus (HBV) positive subjects with liver cirrhosis as well as with different grade of Child-Pugh and 37 healthy subjects were included in this study. The oral glucose tolerance test (OGTT), C-peptide and insulin release test were detected. Plasma glucose and insulin levels were analyzed periodically for 2 h after oral glucose loading. Results: There was no significant difference in the level of fasting plasma glucose and C-peptide between cirrhosis group and control group ($P>0.05$). The levels of OGTT 2 h glucose, insulin and C peptide were significantly higher in cirrhosis group than control group ($P<0.01$). Peak plasma glucose levels were obtained at 60 min in normal group and cirrhosis group. The peak insulin and C-peptide response occurred at 60 min in normal group, whereas it was delayed to 120 min in cirrhosis group. There was a significant difference between two groups in the pattern of plasma glucose levels at corresponding time points ($P<0.05$). The OGTT 2 h glucose and insulin levels were positively correlated with Child-Pugh Score ($r1 = 0.389$, $r2 = 0.508$, $P<0.01$). Conclusion: These findings implied that there was a certain degree of insulin resistance and abnormal glucose metabolism in the patients with liver cirrhosis.

Keywords: Glucose metabolism, insulin secretion, hepatitis virus, liver cirrhosis, oral glucose tolerance test

Introduction

The liver is the vital organ of body to maintain a relatively stability of the blood sugar levels, which has the capability of glycogen synthesis and decomposition. There is abnormal glucose metabolism when the liver cell damage occurs in the patients with cirrhosis. Hepatitis virus B (HBV) is the more common one of the causes of chronic liver diseases worldwide [1]. Chronic hepatitis B is an insidiously progressive form of liver disease, and it does not relentlessly but silently to cause cirrhosis and/or hepatocellular carcinoma over a period of 10-30 years. Recently, it have been reported that cirrhotic patients had a high prevalence of DM, in which most were associated with HBV/HCV infection. And the chronic HBV infection was associated with type 2 diabetes mellitus (T2DM) [2]. In cirrhotic patients with DM, the main cause of death is hepatic failure rather than the complications of DM. Moreover, it has been suggested that T2DM could promote the development of HCC and poor prognosis of liver transplantation. Thus, early intervention was necessary to prevent or improve T2DM to get good prognosis in HBV patients [3].

The 20% to 50% patients with liver cirrhosis showed impaired glucose tolerance and diabetes mellitus (DM). It has been suggested that the presence of DM had relationship with a poor prognosis in cirrhotic patients over a long follow up period [4]. It was still uncertain whether there is the prognostic significance of the presence of DM in cirrhosis. While strictly controlling blood glucose was crucial to the prognosis of diabetic patients [5]. However, glycaemic control is difficult in cirrhotic patients due to the malnutrition and their short life expectancy. Besides, several reports but no clinical data described the relationship between glycaemic control and the prognosis of cirrhotic patients.

On the basis, the aim of this study is to investigate the glucose metabolism and insulin secretion in subjects of chronic hepatitis B with cirrhosis. For this purpose, the level of fasting

Table 1. Demographical, laboratory, metabolic, virological and histological features of 106 patients with chronic hepatitis B

Variable	CHB with cirrhosis	Child-Pugh A	Child-Pugh B	Child-Pugh C	Control	*P* value
Mean age (years)	47.5±16.8	44.9±15.4	47.1±16.5	49.2±17.3	46.7±14.9	0.12
Male/female	81/25	27/8	23/7	31/10	29/8	0.45
Log10HBV-DNA	5.8±1.2	6.1±1.4	5.7±1.6	5.8±1.7	-	-
Mean body mass index (kg/m^2)		22.5±2.1	21.3±1.9	20.9±2.3	23.1±2.3	0.30
Alanine aminotransferase		56.3±25.2	72.6±30.0	96.4±55.8	25.1±13.6	0.36
Cholesterol (mmol/L)		3.3±2.4	3.1±2.6	2.9±2.1	3.8±2.9	0.18
Triglycerides (mmol/L)		1.3±0.4	1.2±0.5	1.1±0.7	1.3±0.6	0.13

Data are means ± SD.

Table 2. The level of fasting plasma glucose, plasma insulin, C-peptide in different classification of Child-Pugh liver cirrhosis

Groups	n	Glucose (mmol/L)	Insulin (mU/L)	C-Peptides (pmol/L)	HOMA-IR	Insulin secretion
Cirrhosis	106	4.65±1.74	19.27±6.21##	490±146	3.98±1.89##	25.43±10.55##
Child-Pugh A	35	4.37±1.76	15.59±5.37#	475±142	3.03±1.45#	30.47±12.34#
Child-Pugh B	30	4.50±1.69	16.71±5.75#	483±137	3.25±1.58#	28.9±10.68#
Child-Pugh C	41	4.90±1.88	22.59±7.16##	511±149	4.92±2.35##	22.62±8.73##
Normal	37	4.29±1.68	10.81±4.46	448±131	2.06±0.98	41.44±16.37

Data are means ± SD, #P<0.05; ##P<0.01 (compare with the normal group).

plasma glucose, C-peptide, HOMA-IR, insulin secretion, OGTT, OGTT 2 h glucose, insulin, C peptide were compared between cirrhosis group and the control group. The strict blood glucose control and infection control might be very important in prolonging the survival in compensated cirrhotic patients with DM.

Materials and methods

Subjects

106 HBV-infected individuals with cirrhosis and 37 healthy donors were enrolled in this study. The HBV-infected patients with cirrhosis were diagnosed with liver cirrhosis by laparoscopic and/or histological findings from December 2007 to January 2012 in the Department of Jiangyin Hospital Affiliated to Southeast University. There are 35 cases Child-Puge A, 30 cases Child-Puge B, 41 cases Child-Puge C in patients with cirrhosis. 25 of 41 cases liver cirrhosis with Child-Puge C conducted a second OGTT test after their condition improved (interval of at least half year ago). We obtained the consent of the blood donors or their guardians in a manner consistent with the policies of the appropriate local institutions. HBV-negative controls were healthy volunteers who were age-, sex-, body mass index, and ethnically-matched with cirrhosis group. A short history was obtained from all normal donors to ensure that they had no infectious disease in the past 3 months.

Clinical evaluation

All patients received comprehensive clinical and laboratory assessment. A 12-h over night fasting blood sample was collected at the time of biopsy to determine serum levels of ALT, total cholesterol, triglycerides and plasma glucose concentration. HBV-DNA was quantified by bDNA test. (Versant HBV3.0, Siemens Medical Solutions Diagnostics Europe, Dublin, Ireland; range 357 (R) C17857000 IU/ml).

The fasting plasma glucose, plasma insulin and C-peptide of blood samples were detected. Serum glucoses were determined by standard laboratory techniques in clinical. Serum insulin and C-peptide was determined using two-site Enzyme ELISA (Mercodia Sweden).

Insulin resistance was determined by the homeostasis model assessment (HOMA-IR) according to the formula: HOMA-IR [fasting glucose (millimoles per liter) × fasting insulin (milliunits per liter)]/22.5. Insulin secretion was assessed using the C-peptide/insulin ratio. Both models have been previously validated against clamp measurements [6, 7].

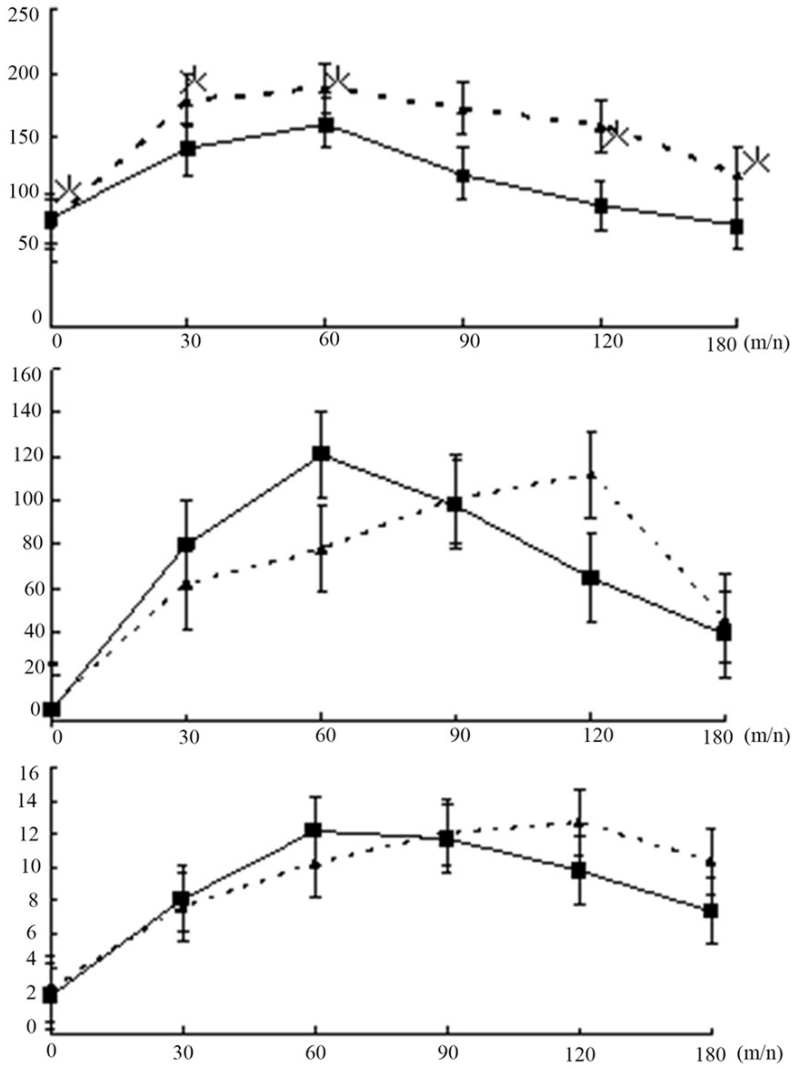

Figure 1. Plasma glucose (top panel), insulin (middle panel) and C-peptide (bottom panel) levels after an oral load of 75 g glucose in cirrhosis group (▲) and normal group (■). Data are mean ± standard deviation. *P<0.05; cirrhosis group vs. normal group.

Blood samples were collected at 0, 30, 60, 90 and 120 min for OGTT with 75 g of glucose; hepatic insulin extraction was assessed by calculating the molar ratio between C-peptide and insulin in the fasting state and at 2 h after OGTT and by the molar ratio of the incremental areas over 2 h between C-peptide and insulin. Total insulin and C-peptide secretion were calculated as the total area under the response curve for 2 h after OGTT.

Statistical analysis

The test results were expressed as mean ± standard deviation. The single-factor analysis of variance and linear correlation analysis was conducted using SPSS 10.0 statistical software, and P<0.05 was considered statistically significant.

Results

The clinical and bio-chemical features of patients in the study were shown in **Table 1**. Both groups were well-matched for age, sex, BMI, log10 HBV-DNA, alanine aminotransferase, cholesterol and triglyceride levels. There was no significant difference in these different characteristics among patients with chronic hepatitis B.

Old life expectancy. Besides, the results of fasting plasma glucose, plasma insulin, C-peptide in different classification of Child-Pugh liver cirrhosis were shown in **Table 2**. There was no significant difference in the level of fasting plasma glucose and C-peptides between cirrhosis group and normal group. While there was a statistical significance in fasting plasma insulin between cirrhosis group and normal group (P<0.05). Besides, the cirrhosis group had statistically significant differences in HOMA-IR and insulin secretion comparing with normal group (P <0.05).

Results of oral glucose tolerance test were shown in **Figure 1**. Peak plasma glucose levels were obtained at 60 min in two groups (normal group and cirrhosis group). The peak insulin response occurred at 60 min in normal group, whereas it was delayed to 120 min in cirrhosis group. And the response pattern of C-peptide was similar to insulin, in which the curve reached the peak levels at 60 min in normal group and at 120 min in cirrhosis group. There was a significant difference between two groups in the pattern of plasma glucose levels at corresponding time points (P<0.05).

Table 3. The level of 2 h OGTT plasma glucose, plasma insulin, C-peptide in different classification of Child-Pugh liver cirrhosis

Groups	n	Glucose (mmol/L)	Insulin (mU/L)	C-Peptides (pmol/L)
Cirrhosis	106	8.09±2.71##	71.87±27.51##	2661±853##
Child-Pugh A	35	6.91±2.17#	60.13±21.84##	2621±827##
Child-Pugh B	30	7.68±2.35#	63.89±23.21##	2597±815##
Child-Puge C	41	9.69±3.15##	82.97±33.64##	2759±902##
Normal	37	5.79±2.01	40.11±15.65	1347±501

Data are means ± SD, #P<0.05, ##P<0.01 (compare with the normal group).

Table 4. Comparison of plasma glucose, plasma insulin, C-peptide on different classification of Child-Pugh and different time in 25 cases liver cirrhosis

Child-Pugh	Glucose (mmol/L)	Insulin (mU/L)	C-Peptides (pmol/L)
The level of fasting			
Child-Pugh C	4.83±1.79	21.97±7.23	499±153
Child-Pugh B/A	4.47±1.69	17.13±6.47#	481±137
The level of 2 h OGTT			
Child-Pugh C	9.73±3.26	83.58±32.47	2803±887
Child-Pugh B/A	8.12±2.73#	69.45±26.42#	2672±867

Data are means ± SD, #P<0.05 (self-comparison).

The levels of OGTT 2 h glucose, insulin, C peptide were significantly higher in cirrhosis group than the control group (P<0.01), which were shown in **Table 3**.

There was no significant difference in the plasma glucose, plasma insulin and C-peptide among different classifications of Child-Pugh and different time (P>0.05), and results were shown in **Table 4**. While, there were significant differences between Child-Pugh B/A and Child-Pugh C in fasting insulin, OGTT 2 h glucose and insulin (P<0.05). Furthermore, the OGTT 2h plasma glucose and insulin were positively correlated with the Child-Pugh Score (r1 = 0.389, r2 = 0.508, P<0.01).

Discussion

Steatosis and insulin resistance (IR) are the key conditions in patients with nonalcoholic fatty liver disease (NAFLD). Hepatic fat accumulation increases oxidative stress resulting in inflammation and fibrosis [8-10]. Large community-based studies have suggested that the prevalence of DM is much higher in HCV-infected patients than the general population as well as patients with other chronic liver diseases such as HBV and alcoholic liver disease [11]. Liver cirrhosis is a chronic disease based extensive damage, and easy to lead to abnormal glucose metabolism [12]. Previous studies showed that 60-80% of patients with cirrhosis occurred impaired glucose tolerance; about 10-15% of them might eventually develop into diabetes [13]. In our study, 67 cases of liver cirrhosis patients showed abnormal glucose tolerance, and 14 patients were diagnosed with diabetes. We also found that the abnormal glucose tolerance level was significantly higher in Child-Pugh C cirrhosis patients than Child-Pugh A grade and B grade group. Child-Pugh score was positively correlated with the OGTT 2 h glucose level.

In conclusion, DM was considered as a comorbid disease in HBV-related cirrhosis, and strict control of blood glucose levels could improve the survival rate of patients with HBV-related cirrhosis [14, 15]. We suggested that individualized assessment of therapeutic benefits should be considered for patients with cirrhosis.

We also found that the Child-Pugh score were positively correlated with insulin levels in the patients with cirrhosis (P<0.01), and prompted Child classification was associated with insulin resistance. Insulin resistance was more obvious in patients with severe cirrhosis and liver damage. The possible mechanisms of insulin resistance in cirrhosis were as follow: (1) hyperinsulinemia: liver dysfunction in cirrhosis patients with insulin degradation could reduce portal blood flow of the liver cells, and reduce the uptake of insulin further then result in hyperinsulinemia. Data showed that shunt might improve glucose tolerance in patients after blocking. (2) insulin receptor abnormalities: impaired glucose tolerance have been reported in the cirrhosis patients. The number and affinity of insulin receptors of liver cells, muscle cells, fat cells decreased, which weakened the effects of peripheral insulin and caused the formation of insulin resistance; In addition, it was reported that serum soluble tumor necrosis factor receptor levels was higher in liver cirrho-

sis patients with insulin resistance, which suggested that tumor necrosis factor system might play an important role in the regulation of insulin activity.

C-peptide could directly reflect the islet β cell insulin production and secretion capacity, and it was barely affected by the liver and kidney function. Some researchers held that cirrhosis was a systemic disease, and hepatitis B virus might invade the pancreas, but there was no direct evidence that HBV could induce insulin secretion disorders. The results indicated that there was no significant difference in fasting C-peptide levels between patients with cirrhosis and control group. But glucose and C-peptide levels were significantly higher in cirrhosis group than normal group. Those suggested that insulin islet β-cell function had no significant obstruction, and viral factor was not an important factor of glucose metabolism disorders in cirrhosis patients.

In addition, the study found that there was no significant difference in the level of fasting glucose between cirrhosis group and control group, which reason might be that it was related with the decreased of hepatic glucose output. Therefore, patients with cirrhosis should be checked their fasting glucose and given OGTT test routinely to find glucose tolerance or diabetes early.

Besides, we also found that the peak insulin and C-peptide response occurred at 60 min in normal group, whereas it was delayed to 120 min in cirrhosis group. And there was a significant difference among two groups in the pattern of plasma glucose levels at corresponding time points ($P<0.05$) (**Figure 1**). And HOMA-IR in patients with chronic hepatitis B and cirrhosis was significantly higher, while their insulin secretion was higher than normal group. Insulin resistance increased with the development of glucose intolerance and the increase of cirrhosis degree (**Table 2**). These findings indicated that the glucose metabolism was abnormal in the patients with cirrhosis. And insulin resistance might play an important role in determining the magnitude of the pathogenesis of glucose intolerance.

In conclusion, there is a certain degree of insulin resistance and abnormal glucose metabolism and aggravation of liver damage in patients with liver cirrhosis. Therefore, it is very important to protect liver function early and effectively to prevent abnormal hepatic glucose metabolism.

Disclosure of conflict of interest

None.

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