

CLINICAL RESEARCH

Biomarkers of Liver in Patients with Chronic Pancreatitis Associated with Metabolic Syndrome

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Abstract

A study of the carbohydrate and lipid metabolism, as well as the leptin level was conducted in 69 patients with chronic pancreatitis (CP) associated with the metabolic syndrome (MS). The results revealed significant changes in the serum parameters studied in patients with CP and MS. The authors concluded that in such patients, the irregularities in the glucose-insulin homeostasis are evident, not only due to hormonal disorders, but also due to impairment cellular metabolism, against the backdrop of excessive free fatty acid (FFA) levels.

Keywords: chronic pancreatitis; metabolic syndrome; carbohydrate and lipid metabolism.

Introduction

Chronic pancreatitis (CP) occupies one of the leading positions in the pathology of the digestive system. The significant prevalence of CP is determined by the central role of the pancreas in the organization of cavitory digestion. A lesion in the exocrine region of the pancreas in CP patients invariably involves the endocrine portion in the pathological process with the development of insulin resistance (IR) and diabetes mellitus [1,2].

The main components of the cascade of metabolic abnormalities in MS are closely related to the functional state of the digestive system. Eating disorders, hormone imbalances of the digestive tract as well as functional condition of the liver and pancreas are key factors in the development of MS [1,3,4,5].

Despite significant advances in the study of the pathogenesis of isolated CP and MS, as well as insulin resistance, the CP and MS combination results in many white spots, the solution for which is important for the timely diagnosis and treatment of this condition.

The aim of our study was to determine some features of the metabolic disorders observed in the livers of patients with CP combined with MS.

Material and Methods

The study included 69 patients (14 men and 55 women; mean age 52.2 ± 2.6 years), all of whom were referred for inpatient treatment, with a diagnosis of CP. The control group included 14 healthy subjects without any objective manifestations of CP and MS.

Verification of the CP was conducted, based on clinical, laboratory and instrumental investigations, taking into account the recommendations of The European Pancreatic Club. The activity of the inflammatory process was evaluated based on the blood tests, serum and urine amylase activity, C-reactive protein, alanine / aspartate transaminases (ALT and AST) and fecal elastase activities. To determine the possible cholestasis, total and direct bilirubin, alkaline phosphatase (ALP) and gamma-glutamyl transpeptidase (GGT) were studied. Serum total protein, albumin, cholesterol, triglycerides, low-density and very low-density lipoprotein cholesterol, absolute lymphocyte count were studied for evaluation of the patient's nutritional status. To assess the changes of the exocrine pancreatic function, a coprogram was performed in all the patients. To optimize the research and identification of latent

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steatorrhea, enzymes were canceled and three days before the study, the patients received a diet with 70-80g fat per day.

Furthermore, a transabdominal ultrasound was performed using «Philips-SD- 360». The size and the acoustic structure of the pancreas, liver, gallbladder, spleen, the state of the portal system vessels and the mesenteric vessels were assessed. The condition of the pancreatic and bile ducts was carefully studied.

MS was diagnosed according to the criteria recommended by the experts of US National Cholesterol Education Program (Update of the Adult Treatment Panel III Guidelines, 2004). The criteria for MS included a waist circumference greater than 102 cm in men and more than 88 cm in women, blood pressure of 130/85mmHg and above, and plasma glucose levels in the blood glucose, at 5.6 mmol/l or more, blood serum triglyceride content of 1.7 mmol/l or more and HDL-C of less than 1 mmol/L for men and less than 1.3 for women.

Patients were informed about the details of the ongoing study and signed an agreement to participate in the research.

The blood glucose test was performed on a fasting basis (fasting blood glucose, FBG) using «HUMAN» sets. The fasting serum insulin level was measured by ELISA («DBG - Diagnostics»). Insulin resistance status was calculated using the homeostatic model assessment-insulin resistance (HOMA-IR). The calculation formula employed is as follows:

$HOMA-IR = (\text{fasting insulin } [\mu\text{IU/ml}] \times \text{fasting blood glucose } [\text{mmol/l}]) / 22.5$.

Patients with a HOMA index above 2.27 were considered IR patients.

The blood leptin level was examined by ELISA using the «BioKhimMak» sets (Russia). Serum FFA concentrations were assayed using a NEFA FS kit (DiaSys, Holzheim, Germany) according to the manufacturer's protocol.

The total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG) levels were determined in the venous blood using «Roche Reflotron Plus» analyzer (Germany). Low-density lipoprotein cholesterol (LDL-C) was calculated according to Fridvald's formula.

As a marker of the cytoplasmic enzymes, the fructose 1-phosphate aldolase (FPA) and fructose-1,6-bisphosphate aldolase (FBPA) activities were determined by the V. Tovarishskiy method (modification of I. Broginskiy), as well as the general activity of the lactate dehydrogenase and gamma-glutamyl transpeptidase were determined using the set «HUMAN». As a marker of the mitochondrial enzymes, the malate dehydrogenase (MDH) activity (optical method of Warburg) was determined.

For the study, the biochemical analyzers («HOSPITAX» and «HUMAN») were used.

The study was approved by the Tashkent Institute of Postgraduate Medical Education Ethics Committee.

Statistical analysis was performed using the statistical software «Statistica -6.1». The mean (M) and standard error of the mean were deduced. Analysis of the distribution of values obtained was performed using the Kolmogorov-Smirnov test. For data with normal distribution, significance was assessed using Student's t-test. A value of $P < 0.05$ was considered statistically significant.

Results and Discussion

The results obtained indicated the presence of significant changes in the blood parameters studied in patients with CP and MS (Table 1). The changes detected in the blood lipid spectrum were accompanied by increased FFA levels on an average of three times and hyperinsulinemia. The effect of lipotoxicity accompanied by hyperinsulinemia and hyperglycemia was associated with the dynamics of the increase in the blood leptin levels. According to many authors, leptin stimulates the proliferation of the β -cells of the islets of Langerhans by phosphorylation of the mitogen-activated protein kinase and correlates with body mass index.

The studies did not reveal any significant changes in the biochemical parameters of CP patients compared with healthy individuals. Disorders of carbohydrate and lipid profiles in patients with CP and MS were associated with the activation of lipolysis. Obviously, an impairment of a receptor-mediated transport of FFAs against the background of hyperinsulinemia leads to structural changes in the cell membrane which, naturally, launches a vicious cycle of metabolic changes in the body cells.

The complex of systemic metabolic impairments discussed above primarily occurs in the liver hepatocytes. Under these conditions, glucose oxidation in the mitochondria is disturbed due to the membrane unresponsiveness to the hormone resulting in pathological insulin resistance syndrome. In this situation, the congestion of the cells with FFA is one of the causes of overproduction of the reactive oxygen species. Increased production of the reactive oxygen species or oxidative stress, further affects the degradation of the biological membranes, particularly the mitochondrial membranes. High permeability of the mitochondrial membranes against the background of the generation of the reactive oxygen species leads to electron leakage, as well as the exit of the marker enzymes from the mitochondria. As seen from the results (Table 1), the serum levels of mitochondrial malate dehydrogenase exceeded the control level by 2.5 times. The condition of hypertriglyceridemia, hyperglycemia, hyperinsulinemia and hyperleptinemia, against the background of high levels of FFA, activates gluconeogenesis and inhibits glycolysis.

Table 1

Blood parameters in patients with CP and MS

Parameters	Control group (n=14)	CP patients (n=17)	CP+MS patients (n=52)
Leptin, ng/ml	13.94±0.75	14.01±0.54	20.42±1.46*
FFA, mmol/l	0.33±0.02	0.72±0.11	0.98 ±0.14*
FPA, IU/l	3.34±0.22	5.01±0.47	40.57±1.27*
FBPA, IU/l	3.62± 0.44	9.06±0.89*	164.3±7.51*
MDH, IU/l	88.4±6.33	101.4±11.8	214.8±11.02*
TC, mmol/l	4.51±0.37	5.08±0.43	5.82±0.34*
TG, mmol/l	0.93±0.08	1.14±0.12	1.97±0.11*
FBG, mmol/l	4.66±0.32	5.01±0.37	5.68±0.21*
Postload glucose, mmol/l	5.08±0.43	5.24±0.41	6.81±0.49*
Fasting insulin, $\mu\text{IU/ml}$	10.72±0.81	11.08±0.86	16.24±0.93*
HOMA-IR	2.21±0.13	2.46±0.11	4.09±0.31*

*- $p < 0.05$ vs control

The fructose-1,6-bisphosphate aldolase participates in the transformation of fructose-1,6-bisphosphate into fructose 6-phosphate and then step by step into glucose, which is one clear marker of gluconeogenesis. As evident from the data, the serum activity of fructose-1,6-bisphosphate aldolase in patients with CP and MS exceeded the reference level by 4.5 times. The fructose 1-phosphate aldolase activity involved in the fructose metabolism exceeded the control level by 12 times and was associated with excessive FFA production. Thus, in patients with CP and MS, the irregularities in the glucose-insulin homeostasis are evident, not only due to hormonal disorders, but also due to impairment cellular metabolism, against the backdrop of excessive FFA levels

Findings

1. The condition of hypertriglyceridemia, hyperglycemia, hyperinsulinemia, and hyperleptinemia against the background of a high FFA level activates gluconeogenesis and inhibit glycolysis.

2. In patients with CP in combination with MS, impairments in the glucose-insulin homeostasis were observed to result not only from hormonal disorders, but also from impairments in cell metabolism against the background of excessive FFA levels.

Competing interests

The authors declare that they have no competing interests.

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