Serum hepatocyte growth factor as an index of extensive catabolism of patients awaiting liver transplantation

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Abstract

Background—Whole body catabolism as the result of intrahepatic metabolic derangement is common in liver transplant candidates. However, individual nutritional assessment parameters lack sensitivity and specificity in determining energy status of these patients. Recently, serum hepatocyte growth factor (HGF) has been shown to reflect the recovery of hepatic energy metabolism after liver transplantation.

Aims—The relation between preoperative levels of serum HGF and metabolic variables was investigated to clarify the clinical value of measuring HGF in evaluations of the catabolism.

Patients/Methods—Blood samples were obtained from 30 liver transplant recipients, and biopsy specimens were taken from each recipient’s rectus muscle and the explanted liver. Preoperative serum concentration of HGF was determined. Whole body energy metabolism was assessed by measuring glycogen contents of biopsy specimens and plasma or serum levels of glucose, insulin, total ketone bodies, total carnitine, and amino acids.

Results—Serum HGF concentration was elevated in 22 of 30 patients and correlated with the Child-Pugh score. It showed a negative association with muscle glycogen content, and a positive correlation with serum levels of glucose, total carnitine, and total ketone bodies. Patients with elevated serum HGF concentrations had higher preoperative plasma levels of aromatic amino acids and branched chain amino acids, associated with lower branched chain to aromatic amino acid ratios.

Conclusions—The elevated serum concentration of HGF in liver transplant candidates reflected inhibition of peripheral glucose storage, enhanced lipid oxidation, and increased peripheral release of branched chain amino acids, and thus extensive energy catabolism.

Keywords: hepatocyte growth factor; catabolism; liver; transplantation

Hypermetabolism associated with protein-energy malnutrition is present in a significant proportion of liver transplant candidates and contributes to systemic manifestations of negative energy balance and thus catabolism. As the early postoperative period after liver transplantation is characterised by a state of hypermetabolism associated with hypercatabolism, and as these aspects of metabolic deterioration have been recognised as risk factors for increased postoperative mortality and morbidity, preoperative assessment of energy catabolism would be useful for the care of liver transplant recipients.

However, malnutrition has not been quantified previously in the adult transplant population; the definition or assessment of metabolic status in patients with end stage liver disease has been an obstacle. Measurement of visceral proteins, including albumin, transferrin, retinol binding protein, and prealbumin, provides limited information for nutritional assessments, particularly those of patients with liver disease, in whom the concentrations may reflect reduced hepatic synthesis or the degree of liver damage rather than nutritional status. Owing to excess fluid accumulation, some anthropometric measures are less sensitive for defining malnutrition.

Hepatocyte growth factor (HGF) is the most potent stimulator of hepatocyte growth and DNA synthesis. It is produced by non-parenchymal hepatic cells and acts as a growth factor, interacting with specific membrane receptors on the surface of hepatocytes to induce signal transduction. It plays a key role in the regulation of liver regeneration after partial hepatectomy or hepatocyte damage. Recently, serum HGF has been reported to be a possible index of catabolism in cancer patients. Moreover, another study showed that the clearance of serum HGF after transplantation reflects the recovery of hepatic energy metabolism as well as graft viability. As intrahepatic metabolic changes reflect the whole body catabolic state and can be seen as being adaptive for energy homeostasis, preoperative serum HGF levels may reflect the whole body energy status of patients awaiting liver transplantation.

This study was designed to evaluate the relation between preoperative serum HGF levels and the metabolic status of liver transplant candidates and to clarify the clinical value of measuring HGF for evaluating the deterioration of whole body energy metabolism.

Abbreviations used in this paper: HGF, hepatocyte growth factor; A, aromatic amino acid; BCAA, branched chain amino acid.
Hepatocyte growth factor and catabolism in liver transplant recipients

Patients and methods
A total of 30 recipients who had undergone orthotopic liver transplantation at The Queen Elizabeth Hospital were enrolled in the study. Twenty normal healthy age and sex matched volunteers were used as controls. The transplants were performed in patients with chronic liver disease (primary biliary cirrhosis in 15, hepatitis C virus cirrhosis in five, cryptogenic liver cirrhosis in four, α-1-antitrypsin deficiency in two, cystic fibrosis in two, extra-hepatic biliary atresia in one and cystic biliary disease in one). The mean age of the patients was 48 years (range 26–69). The Child–Pugh score was obtained by summing up the points for the following variables: (a) ascites (none = 1, slight = 2, moderate to severe = 3); (b) encephalopathy (none = 1, grade I or II = 2, grade III or IV = 3); (c) serum bilirubin (mmol/l) (0–34 = 1, 35–51 = 2, > 52 = 3); (d) serum albumin (g/l) (> 36 = 1, 28–35 = 2, < 28 = 3); (e) prothrombin time (seconds) (< 15 = 1, < 20 but ≥ 15 = 2, ≥ 20 = 3). The livers were preserved for 13.6 (0.4) hours (mean (SEM); range 9.5–17 hours) in University of Wisconsin solution. During surgery and for the first 24 hours after surgery, lactated Ringer’s solution and blood products were administered as needed. A 5% glucose solution was administered on post-operative days 1 and 2. Insulin was required in a few patients after day 1. All patients received standard immunosuppression treatment consisting of low dose predonisolone and cyclosporine or Tacrolimus.

Arterial blood samples were obtained through a catheter inserted for monitoring blood pressure before the operation. The samples were collected in tubes kept on ice and centrifuged at 0°C, and the serum and plasma were stored at −70°C until assayed. Blood samples were obtained from the 20 healthy controls for HGF concentration analysis. Serum HGF concentrations were determined using an enzyme linked immunosorbent assay kit (Otsuka Assay, Tokushima, Japan). Glucose concentrations were determined on a neutral-detergent based enzymatic method using highly purified 3-hydroxybutyrate dehydrogenase, based on the methods of Melanby and Williamson.14 Plasma concentrations of total carnitine were determined by a radioenzymatic assay (Kainos, Tokyo, Japan). Plasma amino acid concentrations were determined in plasma deproteinised with 10% sulphosalicylic acid using high performance liquid chromatography on an amino acid analysing system JLC automatic analyser (Nihon Denshi, Tokyo, Japan). The following were determined: aromatic amino acids (AAAs; tyrosine + phenylalanine), branched chain amino acids (BCAAs; valine + isoleucine + leucine), and the BCAA to AAA molar ratio. Arterial blood samples were also obtained during the anhepatic phase just before graft reperfusion for analysis of plasma amino acid concentrations.

A biopsy specimen was taken from each recipient’s rectus muscle at the incision site of the operative wound. A wedge biopsy specimen was taken from the recipient’s explanted liver soon after its removal. The wet weights of the biopsy specimens were immediately recorded. The specimens were then immediately frozen in liquid nitrogen and stored at −70°C until assayed. The glycogen contents of these specimens were determined as follows. Briefly, the specimens were homogenised with amyloglucosidase after correction for tissue free glucose, and the increase in NADPH was then measured fluorometrically.

Informed consent to participate in the study was obtained from each subject. The results are presented as means (SEM). Differences in HGF concentration between patients and controls for HGF concentration analysis.
trols were assessed using the Mann-Whitney U test. In a univariate analysis, preoperative levels of HGF and various variables were analysed. Intragroup differences were compared using the Wilcoxon signed rank test. \( p<0.05 \) was considered to be significant.

**Results**

The mean serum HGF concentration of the patients was significantly higher than that of the controls (0.82 (0.19) \( \text{ng/ml} \) vs 0.17 (0.01) \( \text{ng/ml} \), \( p<0.0001 \)). The cut-off value was set at 0.5 \( \text{ng/ml} \) based on control values (>mean + 4 SD). Serum HGF concentration was elevated in 22 of 30 patients (73%). One patient whose preoperative HGF level was the highest of all the patients (7.49 \( \text{ng/ml} \)) died 38 days after the transplantation with multiorgan failure resulting from initial poor graft function. Two patients with serum HGF concentrations above 1.0 \( \text{ng/ml} \) had a septic complication. The other 27 patients survived without morbidity. Serum HGF concentration correlated with the Child-Pugh score (fig 1).

Figure 2 shows the relation between serum HGF concentration and glycogen content of muscle and explanted liver. The normal range of glycogen content of skeletal muscle is 10–60 mg/g muscle. \( ^{15} ^{16} \) Serum HGF concentration correlated with muscle glycogen content, but not with the glycogen content of the explanted liver.

Figure 3 shows the relation between serum HGF concentration and each of the variables reflecting energy metabolism of the patients. Serum HGF concentration correlated with serum levels of glucose, total ketone bodies, and total carnitine.

Figure 4 shows the relation between serum HGF concentration and preoperative plasma levels of branched chain amino acids (BCAA) (A), aromatic amino acids (AAA) (B), and the BCAA to AAA molar ratio (C) in patients awaiting liver transplantation (\( n = 30 \)).

Serum HGF concentration correlated positively with plasma levels of AAs and BCAAs, and showed a weak negative association with the BCAA to AAA molar ratio.

The plasma BCAA level in patients whose HGF level was above or equal to the cut-off value showed a further increase during the anhepatic phase (379.1 (21.0) \( \text{nmol/ml} \) vs 441.0 (32.0) \( \text{nmol/ml} \), \( p = 0.0142 \)), whereas the plasma BCAA level in those whose HGF level was below the cut-off value decreased significantly at the end of the anhepatic phase (359.8 (34.8) \( \text{nmol/ml} \) vs 311.4 (37.9) \( \text{nmol/ml} \), \( p = 0.0180 \)). The preoperative serum HGF concentration correlated significantly with the plasma BCAA level at the end of the anhepatic phase (fig 5).

**Discussion**

Serum HGF concentration is increased in a variety of liver diseases in which there is liver regeneration. Cirrhotic patients with modified
Hepatocyte growth factor (HGF) is a multifunctional cytokine that plays a critical role in the proliferation, differentiation, and survival of various cell types, including hepatocytes. It is synthesized primarily in the liver and released into circulation, where it can modulate diverse physiological processes.

In liver transplant recipients, the activity of HGF may reflect a decrease in its clearance by the damaged liver, leading to increased levels in the bloodstream. This increase in HGF concentration in patients with liver disease may be related to both hepatic and extrahepatic factors.

Reduced glycogen stores and deranged lipid metabolism are also common findings in liver disease. For instance, in cirrhotic patients, the glycogen content in tissues is significantly reduced, and the liver's glycogenogenic capacity is impaired. This decrease in glycogen stores contributes to the development of hepatic encephalopathy and other metabolic disturbances.

Ketone bodies, generated from fatty acids in mitochondria, are important fuel sources in peripheral tissues during fasting and starvation. In conditions of metabolic stress, such as liver disease, the liver utilizes ketone bodies more extensively, which might explain the increased plasma ketone body levels observed in patients with chronic hepatitis.

Patients with chronic hepatitis have higher preoperative plasma alanine aminotransferase (AAA) and branched-chain amino acid (BCAA) levels associated with decreased BCAA to AAA ratios. This might indicate impaired muscle protein synthesis and increased peripheral utilization of BCAAs. The BCAA to AAA ratio is a useful index for evaluating the energy catabolism of patients awaiting liver transplantation.

The increased plasma AAA levels caused by increased degradation of muscle protein and decreased metabolism in the liver result in a decreased BCAA to AAA ratio in these patients. The BCAA to AAA ratio is normally 3.0 to 3.5, but in the setting of liver failure, this ratio falls, typically below 2.0. In contrast, increased levels of BCAAs and AAAs were observed in patients with fulminant hepatic failure. Increased plasma BCAA levels associated with hyperaminoacidemia in patients with acute liver failure have been reported to indicate a poor prognosis.

As BCAAs leave the hepatic veins after little or no uptake by the liver cells, and as increased plasma BCAA levels indicate the peripheral release of BCAAs (principally in muscle tissues) prevails over the increase in the peripheral utilization of BCAAs, marked increases in BCAAs and AAAs in the plasma suggest that, at this stage, amino acid metabolism is seriously disturbed not only in the liver but also in other tissues.

In conclusion, the elevated serum concentration of HGF in the liver transplant candidates reflected inhibition of peripheral glucose storage, enhanced lipid oxidation, and increased peripheral release of BCAAs. Serum HGF may be a useful index for evaluating the energy catabolism of patients awaiting liver transplantation.


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