Clinical Biochemistry xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Significance of arginase determination in body fluids of patients with hepatocellular carcinoma and liver cirrhosis before and after surgical treatment

Alicja Chrzanowska, Wojciech Graboń, Magdalena Mielczarek-Puta, Anna Barańczyk-Kuźma *

Chair and Department of Biochemistry, Medical University of Warsaw, 02-097 Warsaw, Banacha 1, Poland

ARTICLE INFO

Article history:
Received 16 January 2014
Received in revised form 27 March 2014
Accepted 28 March 2014
Available online xxxx

Keywords:
Arginase activity
Isoenzymes
Serum
Bile
Hepatocellular carcinoma
Liver cirrhosis

ABSTRACT

Objective: To assess the utility of arginase activity and expression in diagnosis of liver diseases.

Design and methods: Arginase activity, sensitivity and specificity were determined in serum of 140 patients including 50 with HCC, 60 with LC, 30 with choledocholithiasis (CDL) and 90 healthy controls. In HCC and LC arginase activity in serum was studied before and after tumor resection or liver transplantation. Arginase sensitivity in HCC was compared to that of alpha-fetoprotein (AFP) and aminotransferases (AST, ALT). In LC the activity was determined also in bile before and after transplantation. The expression of arginase isoenzymes in serum was studied by Western blotting.

Results: In HCC and LC the preoperative arginase activity was significantly higher compared to controls, and it decreased after surgery. The sensitivity of arginase in HCC was much higher than that of AFP, AST and ALT (96, 40, 20 and 18%, respectively). In HCC it was higher than in LC (93%) and CDL (33%). The specificity of arginase was above 80%. In bile of cirrhotic patients the highest activity was immediately after liver transplantation. It decreased with time but increased dramatically at the time of the graft rejection. Arginase AII was present in serum of HCC and LC but not the control cases.

Conclusions: The increase of arginase activity in serum accompanied by the presence of isoenzyme AII can be useful in HCC and LC diagnosis. The determination of arginase activity in bile may be helpful in monitoring liver graft recipients.

Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer in the world [1,2]. There are wide geographical variations in the incidence of HCC with the highest rate in the developing countries of Asia and Africa, and still increasing incidence in North America and Europe [3]. HCC is one of the few cancers with well-defined major risk factors, among which the liver cirrhosis (approximately 80% of all cases). Other risk factors include hepatitis B and C, aflatoxins and alcoholism. Liver cancer rarely gives symptoms in the early stage and the prognosis is usually poor in advanced stages [4,5]. The development of liver cirrhosis (LC) is also difficult to diagnose since the symptoms are not characteristic and they usually appear late in the course of disease [6,7]. Since HCC is often associated with liver cirrhosis, the diagnosis includes routine laboratory tests assessing liver functions, clotting system, and blood cell count [8]. Aspartate and alanine aminotransferases (AST, ALT), lactate dehydrogenase and alkaline phosphatase are enzymes routinely used as markers of hepatic functions. They are not only localized in the liver, but also in other tissues (skeletal and cardiac muscles, kidney, bones) and released into the circulation during their damage. ALT and AST are sensitive indicators of hepatitis, ischemic or toxic hepatocyte damage, but not liver tumors [9]. There are no highly specific molecular markers of HCC and LC. The most useful HCC marker at present is alpha-fetoprotein (AFP), normally produced by immature liver cells in fetus. The clinical usefulness of AFP is well established in the diagnosis of nonseminomatous germ cell tumors (carcinoma embryonale, Yolk Sac Tumor, chorioncarcinoma, teratoma), as well as in stomach, biliary and pancreatic cancers. Beside tumors, its level is also elevated in pregnancy and noncancerous diseases including ataxia telangiectasia, hereditary tyrosinemia, neonatal hyperbilirubinemia, acute viral hepatitis, chronic active hepatitis and liver cirrhosis [10]. Arginase may be a new and promising marker of HCC and liver cirrhosis. It shows extremely high activity in hepatocytes and is present in two structurally and functionally distinct isoenzymes. Arginase AI, called “hepatic” or “liver-type” is highly expressed in the liver. It is localized in cytoplasm of periportal hepatocytes and participates in ammonia inactivation, as the last enzyme of the urea cycle [11–13]. Arginase AII is expressed in liver mitochondria of perivenous hepatocytes and its activity is much lower than that of AI. It is widely distributed among other tissues, and is called “extrahepatic” arginase. Arginase AII takes part in pathways of intermediary metabolism such as L-proline and polyamine biosynthesis [14,15]. L-Proline is a

* Corresponding author. Fax: +48 22 5720 679.
E-mail address: akuzma@wum.edu.pl (A. Barańczyk-Kuźma).

http://dx.doi.org/10.1016/j.clinbiochem.2014.03.019
0009-9120/

substrate for collagen, the biosynthesis of which increases in liver cirrhosis. Polyamines are important regulators of cell proliferation and differentiation [16,17]. Additionally, arginase (especially AI) may affect NO biosynthesis competing with nitric oxide synthase for the common substrate, L-arginine [18,19]. HCC is resistant to both radiotherapy and chemotherapy, thus the tumor(s) resection is the only method used in its treatment. Considering that liver cirrhosis is an irreversible process, the only effective curative method of the end-stage disease is a liver transplantation [20,21]. Thus the aim of our study was to assess the utility of arginase activity and expression in diagnosis of liver diseases. In the present work we investigated the arginase activity and the profile of its isoenzymes in blood serum of patients with HCC and patients with liver cirrhosis. The studies were conducted before and after tumor/s resection or liver transplantation. The study was broadened by determination of arginase activity in bile of cirrhotic patients at various times after liver transplantation.

Materials and methods

Patients

Blood serum was obtained from 50 patients with hepatocellular carcinoma (HCC) and 60 with liver cirrhosis (LC) qualified for liver transplantation. All patients were treated at the Department of General, Transplant and Liver Surgery and Department of General and Transplantation Surgery, Medical University of Warsaw. The group with HCC included 18 females and 32 males, with a mean age 57.3 ± 9.1 (range 29–73 years; T1-T4N0M0, T3N1M0). The group with LC included 24 females and 36 males, mean age 47.0 ± 8.4 (range 19–63 years). The causes of liver cirrhosis were hepatitis B (n = 16), hepatitis C (n = 11), alcoholism (n = 18), primary biliary cirrhosis (n = 7), and autoimmune hepatitis (n = 8). The patients' diagnosis was made based on an increased level of plasma alpha-fetoprotein (HCC), determination of liver function markers (LC), ultrasonography and computed tomography. All cases were histologically confirmed after the surgery (tumor resection, liver transplantation).

The group of 30 patients with choledocholithiasis (CDL; 14 females and 16 males, mean age 51.79 ± 10.4) was also included into the study. The control group consisted of 90 healthy blood donors matched for age and sex with the groups of patients (32 females and 58 males, mean age 42.0 ± 7.8). Blood from HCC and LC patients was taken 1 day before and 6 days after tumor resection or liver transplantation, respectively. Bile from LC patients was collected from Kehr drain everyday for 2 weeks starting from the first day after liver transplantation. Serum and bile samples were stored at −80 °C for further studies. The studies were approved by the Bioethics Committee of the Medical University of Warsaw, and informed consent was obtained from all patients.

Methods

Arginase activity was determined spectrophotometrically from the amount of ornithine [22], and expressed in U/L of serum or bile. The cut-off value for each group of patients was determined with the use of ROC curve analysis. Protein was assayed according to Bradford [23], with crystalline bovine serum albumin as standards. Alpha-fetoprotein (AFP) level was measured by the Architect® AFP system Chemiluminescent Microparticle Immunoassay (Bio-Rad), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were assayed using Flex® reagent cartridge test (Dade Behring Inc.). The upper limit of normal for AFP was 40 ng/mL, for AST 40 U/L, and for ALT 45 U/L.

Western blotting was performed after electrophoresis in 14% polyacrylamide gel, according to Laemmli [24], with rabbit anti-bovine arginase AI (Res. Diagn. Inc.) and AII (Santa Cruz Biotech. Inc., USA) polyclonal antibodies. Arginase AI from bovine liver and AII from human kidney were used as the standards (Res. Diagn. Inc. and Sigma, respectively). Blots were visualized using ECL plus Western Blotting Detection System (Amersham).

The Shapiro–Wilk test indicated nonnormal data distribution. Comparison of serum arginase activity in patients (HCC, LC, CDL) and the control group was performed by Mann–Whitney *U* test. Wilcoxon test was used for comparison of serum arginase activity in patients before and after surgery, and in bile one and six days after liver transplantation. The statistical calculation was performed using Statistica 10.0 (StatSoft) program. Results were expressed as medians (25th–75th percentile) and considered statistically significant at $P < 0.05$.

Results

Arginase activity in blood serum and bile

The median value of arginase activity in serum of healthy blood donors (control) was 5.6 (4.50–9.12) U/L. In patients with CDL the activity was slightly but significantly higher (11.37 (7.72–15.98) U/L) than in control. It was much higher in HCC and LC patients, with the median value of 37.20 (27.25–43.11) and 32 (27.10–41.38) U/L, respectively (Fig. 1). Six days after tumor resection or liver transplantation arginase activity significantly decreased to 10.79 (8.4–14.26) U/L in HCC and 11.4 (7.73–12.49) U/L in LC patients (Fig. 1).

Arginase activity in bile of patients with LC determined one day after liver transplantation ranged from 65.47 to 777.76 U/L with the median value of 141.30 (91.35–185.50) U/L. In patients without graft rejection the activity decreased gradually and after 14 days ranged from 31.50 to 55.80 U/L (median 44.58 (37.94–54.32) U/L). In bile of 5 patients with graft rejection, arginase activity decreased for a few days and increased importantly at the day of graft rejection (5–9 days after liver transplantation) (Fig. 2). The peak of activity decreased slowly after immunosuppressive treatment. Fourteen days after liver transplantation the activity reached the level similar to that of patients without graft rejection (47.8 (42.75–53.15) U/L) (Fig. 2).

Sensitivity and specificity of arginase versus liver markers

The sensitivity of arginase was 96% for HCC (at cut-off 17 U/L), 92% for LC (at cut-off 16 U/L), and 33% for CDL (at cut-off 13 U/L). The number of not detected cases (false negative, FN) was 2/50 for HCC, 5/60 for LC, and 20/30 for CDL. The specificity of arginase was 98, 95, and 93% in HCC, LC and CDL, respectively. The numbers of detected cases (true negative, TN) were: 88/90 for HCC, 86/90 for LC, and 84/90 for CDL.

The preoperative concentration of AFP in serum of HCC patients ranged from 0.27 to 1750 ng/mL and the activity of aminotransferases — from 10 to 130 U/L (AST) and from 10 to 197 U/L (ALT). The sensitivity

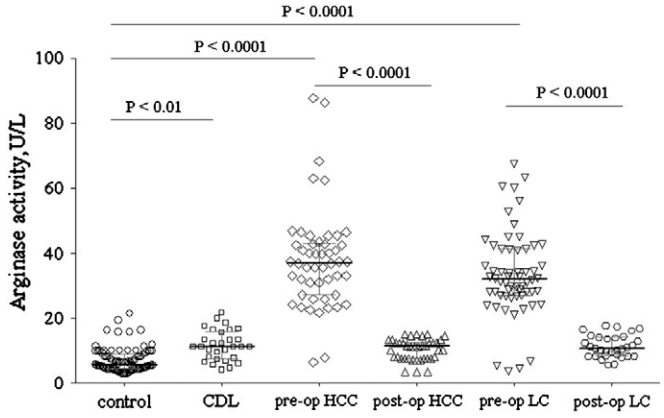

Fig. 1. Arginase activity in blood serum of individual subjects. The activity was determined as indicated in the Material and methods section and expressed as medians. Pre-op, activity 1 day before surgery; post-op, activity 6 days after surgery (tumor resection or liver transplantation).

Please cite this article as: Chrzanowska A, et al, Significance of arginase determination in body fluids of patients with hepatocellular carcinoma and liver cirrhosis before and aft..., Clin Biochem (2014), http://dx.doi.org/10.1016/j.clinbiochem.2014.03.019