CLINICAL STUDY

Serum cholinesterase activity and proteosynthetic function of liver in patients with diabetes mellitus

Turecky L, Kupcova V, Mojto V, Smutny M, Uhlikova E, Vozar I

Department of Medical Chemistry, Biochemistry and Clinical Biochemistry, Comenius University, Bratislava, Slovakia. **laco.t@seznam.cz**

Abstract

Introduction: **Diabetes mellitus is associated with a lot of changes in intermediary metabolism and several authors reported on higher frequency of liver diseases in patients with diabetes.**
Aim of the study: **Was to establish the changes of blood serum cholinesterase, prealbumin and albumin, parameters which are accepted as an index of liver proteosynthetic function, in patients with diabetes mellitus.**
Patients and methods: **The study group consisted of 207 patients with diabetes mellitus (83 patients with type I and 124 patients with type II diabetes mellitus). Control group consisted of 179 healthy subjects. The activity of cholinesterase was assayed by the kinetic method, concentrations of prealbumin and albumin were determined immunochemically.**
Results: **Activity of serum cholinesterase was significantly higher in group of patients with diabetes mellitus than in control group (65.05 vs 73.33 μkat/l). The concentration of prealbumin was lower in blood serum of patients with diabetes than in controls (308.10 vs 285.85 mg/l). Serum levels of albumin were not different in both studied groups. After dividing of patients according to the type of diabetes, 80 % of abnormal values of cholinesterase and prealbumin were present in patients with type II diabetes.**
Conclusions: **The results of our study showed abnormal values of determined liver tests approximately in 22 % of patients with diabetes mellitus. The character of laboratory changes – increased activity of cholinesterase, decreased concentration of prealbumin and normal levels of albumin, suggests development of liver steatosis in these patients. The most of pathological findings were in patients with diabetes type II *(Tab. 3, Ref. 20)*.**
Key words: cholinesterase, prealbumin, diabetes mellitus, liver steatosis, non-alcoholic steatohepatitis, NASH.

Diabetes mellitus is a common condition prevalent in approximately 4 % of Slovak population (Mojto et al, 1997). Diabetes results from abnormal production or use of insulin. There are two aspects to the clinical manifestation of diabetes mellitus, one of which is related to disturbed metabolism and the other to long-term complications of the condition. Diabetes is associated with a lot of changes in intermediary metabolism. Liver plays the central role in metabolic regulation of human body. It is not surprising that diabetes mellitus induces marked metabolic changes in liver tissue potentially leading to disorders of liver functions. Several authors report on high frequency of liver diseases in patients with diabetes mellitus. Toman et al (1981) describes hepatomegaly in 64 % and increased blood serum activities of ALT in 42 % of their diabetic patients. Their morphological analysis shows diabetic steatosis and diabetic microangiopathy in liver biopsies in 40 % of their diabetics. Amarapurkar and Das (2002) describe diabetes as an important risk factor of chronic liver diseases and a progression of non-alcoholic steatohepatitis (NASH) toward cirrhosis.

Serum cholinesterase (CHE, EC 3.1.1.8), also known as pseudocholinesterase, has been recognized as an enzyme that hydrolyzes choline esters. It is generally accepted that serum cholinesterase is produced in the liver and secreted into the blood stream (Brown et al, 1981). Estimation of serum CHE activity

Department of Medical Chemistry, Biochemistry and Clinical Biochemistry, and 3rd Medical Department, Faculty of Medice, Comenius University, Bratislava, Slovakia

Address for correspondence: L. Turecky, MD, PhD, Institute of Medical Chemistry, Biochemistry and Clinical Biochemistry, Medical School, Comenius University, Sasinkova 4, SK-811 08 Bratislava 1, Slovakia. Phone: +421.2.59357576
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Tab. 1. Serum levels of cholinesterase, prealbumin and albumin in patients with diabetes mellitus and in control group.

	Cholinesterase μkat/l	Prealbumin mg/l	Albumin g/l
Controls a=179	65.05±1.11	308.10±3.79	43.42±0.44
Diabetes mellitus n=207	77.33±1.74*	285.85±5.17*	42.18±0.49

Values are given as mean±SEM, *statistically different from control group, $p<0.01$

Tab. 2. Pathological values (increased and decreased) of cholinesterase and prealbumin in patients with diabetes mellitus.

	All pathological values/all patients	Increased values /all pathological values	Decreased values /all pathological values
Cholinesterase	46/207	36/46	10/46
	22.22 %	78.26 %	21.74 %
Prealbumin	47/207	19/47	28/47
	22.71 %	40.43 %	59.57 %

Tab. 3. Pathological values of cholinesterase and prealbumin in patients with diabetes mellitus type I and type II.

	Diabetes mellitus type I pathological values /all patients	Diabetes mellitus type II pathological values /all patients
Cholinesterase	7/83	39/124
	8.4 %	31.5 %
Prealbumin	8/83	39/124
	9.6 %	31.5 %

has been useful in the diagnosis of diseases of the hepatobiliary system because the major portion of plasma CHE is synthesized in the liver and released promptly after its synthesis. Changes in serum CHE activity reflect the changes in hepatocellular functions and have been regarded as sensitive indicators of the diminished synthetic capacity of the hepatic parenchyma (Adolph, 1979). Although the decrease in CHE activity in severe parenchymal liver disease is well known, little attention had been paid to hepatic disorders associated with elevated CHE until a couple of Japanese groups reported on its increase in fatty liver (Shibata et al, 1975; Iwamura et al, 1979). The results of Nomura et al (1986) show that measuring the CHE activity is of diagnostic value and an alternative to computed tomography in hepatic steatosis.

Albumin (MW 66000) is the most abundant protein in human plasma representing 40 to 60 % of the total proteins (Burtis and Ashwood, 1994). The chief biological functions of albumin are to transport and store a wide variety of ligands, to maintain the plasma oncotic pressure and to serve as a source of endogenous amino acids. The plasma albumin concentration is used to test liver functions. Because of its relatively long half-life in the plasma (approximately 20 days), albumin concentration is usually normal in acute liver disorders. Most of chronic liver diseases result in decreased serum albumin levels. The importance of albumin function is the cause of a relatively large functional reserve in the capacity of the liver to synthesise it. This means that diseases of at least moderate severity have a decreasing effect on serum albumin concentrations.

Prealbumin (MW 54000) is another protein synthesized by the liver. It is a negative acute phase protein. Its serum level is significantly decreased in liver dysfunction. Therefore, the serum concentration of prealbumin may serve as an index of liver function. Prealbumin has an extremely short half-life (approximately 1.5 day); consequently, the measurement of its serum levels may provide an assessment of liver dysfunction that is more timely and sensitive than those of albumin and cholinesterase.

The aim of our study was to establish the changes in blood serum cholinesterase, prealbumin and albumin in patients with diabetes mellitus, i.e. in parameters accepted as indexes of the liver proteosynthetic function.

Patients and methods

Subjects. The study group consisted of 207 patients with diabetes mellitus (DM type I – 83 patients, aged 15–64 years, DM type II – 124 patients, aged 31–81 years) without previous anamnesis of chronic liver disease. The control group consisted of 179 healthy subjects (mainly blood donors), aged 18–72 years. The determination of biochemical parameters was performed from samples of blood drawn from the antecubital vein.

Biochemical analysis. The activity of cholinesterase was assayed by the kinetic method of Knedel and Bottger (1967) using butyrylthiocholine iodide as substrate at 25 °C. Serum prealbumin and albumin concentrations were determined by electroimmunodiffusion (Laurell, 1966) using monospecific antisera.

Statistical analysis. Values are given as mean±S.E.M. Differences between group means were tested by the unpaired Student's *t*-test for two-group comparisons, and *p* values less than 0.05 were regarded as statistically significant. Statistical analyses were performed using Statgraphics Plus statistical software Version 5.0.

Results

As shown in Table 1, the activity of serum cholinesterase was significantly higher in the group of patients with diabetes mellitus than that in the control group. The blood serum concentration of prealbumin in patients with diabetes mellitus was lower than that in controls. Serum levels of albumin did not differ in both studied groups. The comparison of prealbumin and cholinesterase levels found in diabetics with the reference values showed pathological values in 22.7 %, or 22.2 % of patients (Tab. 2). We found no pathological albumin values in our patients with diabetes mellitus. The analysis of pathological values

of cholinesterase activity showed that out of 46 patients with pathologically changed values the activity of cholinesterase increased in 36 patients (78.3 %) and decreased in 10 patients (21.7 %). Out of 47 patients with pathological prealbumin concentrations, 19 patients (40.4 %) yielded prealbumin concentrations over and 28 patients (59.6 %) below the reference range. After dividing the patients according to the type of diabetes, abnormal values of cholinesterase were found in 7 patients (8.4 %) with diabetes mellitus type I and in 39 patients (31.5 %) with diabetes mellitus type II (Tab. 3). Abnormalities of blood serum prealbumin concentration were found in 8 patients (9.6 %) with diabetes type I and in 39 patients (31.5 %) with diabetes type II.

Discussion

The results of this study show that biochemical parameters reflecting the liver proteosynthetic function (cholinesterase, prealbumin) were pathologically changed in approximately 20 % of patients with diabetes mellitus. This finding is in accordance with the results of Toman et al (1981) and Araujo et al (1998) who also found abnormalities in the liver function in their patients with diabetes mellitus. The activity of cholinesterase was significantly higher in diabetic patients than in the controls. This observation is analogous to the increased activity of serum cholinesterase reported in rats with experimental diabetes (Desmukh, 1986; Uhlikova et al, 2004). The finding of increased serum cholinesterase activity suggests the presence of liver steatosis in the studied patients with diabetes mellitus and confirms the findings of other authors who describe higher frequency of fatty liver in patients with diabetes mellitus. The most of acute or chronic liver diseases are accompanied with a decrease in the activity of serum cholinesterase. Only non-alcoholic liver steatosis is connected with increased activities of serum cholinesterase (Nomura et al, 1986; Thomas, 2000). Increased activities of cholinesterase were established in 36 out of 46 patients with an abnormal activity of this enzyme. Ten patients had their activities of cholinesterase below the reference range. It is possible that in these patients the disturbance of liver function was more advanced and that simple liver steatosis has progressed toward non-alcoholic steatohepatitis. NASH is a syndrome characterized by its association with fatty liver, lobular hepatitis and chronically elevated alanine aminotransferase plasma levels in patients with negligible alcohol intake (Ludwig et al, 1980). The syndrome is mainly associated with obesity, diabetes and hyperlipoproteinemia (Marchesini et al, 2001). From the clinical point of view, NASH is not a simple harmless liver disorder because ~ 50 % of NASH patients develop liver fibrosis, 15 % develop cirrhosis and 3 % may progress toward terminal liver failure requiring liver transplantation (Sheth et al, 1997). Diabetes mellitus is an important risk factor of the development of NASH. The analysis of a large group of patients with non-alcoholic fatty liver (NAFL) showed that 33 % of these patients had diabetes mellitus. More importantly, diabetes with NAFL had an increased rate of cirrhosis and liver-related deaths (McCullough, 2001). The data from the study of McCullough (2001) strongly suggest that diabetic patients with NAFL are at a higher risk of the development of an aggressive outcome as opposed to other patients with NAFL.

Plasma prealbumin concentrations significantly decreased in the group of patients with diabetes mellitus. The comparison of patient's values of prealbumin concentration to the reference values showed 47 abnormal results (22.7 %). This frequency of pathological values is similar to the frequency of abnormal values of cholinesterase activity, but the proportion of decreased values was greater in the case of prealbumin (59.6 % vs 21.7 %). This great number of pathologically decreased values of prealbumin concentrations in patients with diabetes mellitus could be explained by great sensitivity of prealbumin used as the index of liver proteosynthetic function. The very short half-life of prealbumin with a relatively low reserve of liver capacity to synthesise this protein are the reasons why prealbumin becomes a parameter that indicates liver dysfunction more sensitively than cholinesterase.

There was no change in albumin concentration in the blood serum of patients with diabetes mellitus. This is not surprising because albumin is the most abundant plasmatic protein and has many important functions. This importance of albumin results in a relatively very large functional reserve in the synthesis of this protein within hepatocytes. There may be an extensive reduction in the mass of functioning liver parenchyma before the deficiencies in the synthesis of albumin can be detected. McCullough (2001) also reports normal levels of albumin in patients with NASH.

After dividing the patients according to the type of diabetes, the most of prealbumin and cholinesterase abnormalities were in the group of patients with diabetes type II (approximately 80 % of pathological values). This finding is in agreement with the reports of other authors, who describe the presence of NAFL in patients with diabetes mellitus type II (Yu and Keeffe, 2002; Akbar and Kawther, 2003; Younossi et al, 2004). On the other hand, steatosis is unusual in type I of diabetes (Yu and Keeffe, 2002). The prevalence of NAFL in patients with diabetes type II is reported to range from 25 to 75 % (McCullough, 2002). The relatively low frequency of abnormal laboratory findings in our group of diabetics (46/207, 22.2 %) can be explained by the fact that our group of patients had a high proportion of patients with diabetes type I (~ 40 %), in which liver steatosis is rare.

In summary, the results of our study show abnormal values of determined liver tests approximately in 22 % of patients with diabetes mellitus. The character of laboratory changes – increased activity of plasma cholinesterase, decreased concentration of prealbumin and normal levels of albumin – suggests the presence of liver steatosis in these patients. Decreased cholinesterase activities in the part of patients with abnormal cholinesterase values suggest that in these patients the liver steatosis has progressed toward more severe forms such as steatohepatitis or fibrosis. It is important to remember, mainly in patients with diabetes type II, that liver steatosis, which can progress toward more severe diseases (fibrosis, cirrhosis) can be one of the possible complications of diabetes.

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