ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN-POLONIA
VOL. LX, N 1, 97 SECTIO D 2005

Department of Infectious Diseases, Medical University of Lublin

HANNA FOTA-MARKOWSKA, ROMA MODRZEWSKA,
JOANNA LIS-TØNDER, ROMANA ROLLA-SZCZEPAŃSKA

Dynamics of liver/kidney microsomal antibody type 1 level in serum of patients in acute, symptomatic period of infectious mononucleosis

The Epstein-Barr Virus (EBV) has been classified by the International Committee for Taxonomy of Viruses to *Herpesviridae* family, subfamily of *Gammaherpesviridae*. Viruses of this group cause characteristically latent infections and some of them can act as carcinogens. EBV has been proven to be an etiologic factor of infectious mononucleosis (IM), an acute self-limiting lymphoproliferative disease. EBV belongs to viruses primary hepatotropic and in most patients with IM there are observed clinical, biochemical and histopathological parameters of hepatitis. Mild hepatitis is the rule in infectious mononucleosis and therefore should not be considered a complication. Hepatomegaly occurs in about 15 percent of patients with IM. Studies by many investigators have shown that the vast majority (greater than 90 percent) of patients with IM have abnormal hepatic enzymes. Fulminant hepatitis, hepatic failure with coma and death, or Reye's syndrome has occurred in temporal association with IM (3, 8). Data from literature provide convincing evidence that cytochrome P4502D6 (CYP2D6) is present on the liver cell plasma membrane; also the presence of B cell epitopes on CYP2D6 has been investigated. CYP2D6 is the main target of liver kidney microsomal antibody type 1 (LKM1). LKM1 is not only the serological hallmark of autoimmune hepatitis type 2 but it is also found in up to 10% of patients with chronic hepatitis C virus (4,5). To resolve this problem we have taken approach toward assessing serum LKM1 level in patients with IM.

MATERIAL AND METHODS

The study contained 72 people including 52 patients (29 women and 23 men), being in severe symptomatic period of IM and 20 healthy people (11 women and 9 men). The diagnosis of IM was confirmed by the presence of anti-VCA IgM antibodies in the quantity above 20 AU/ml (ELISA, Organon Technika). Levels of LKM1 in blood serum of patients were measured twice using Enzyme Immunoassay method (Pharmacia Upjohn): on the second day of hospitalisation (study no 1) and after regression of clinical symptoms (study no 2). Obtained data were statistically analysed using Kolmogorov-Smirnov and Cochran-Cox methods.

RESULTS

Using the Kamogorov-Smirnov's test it has been examined that data was distributed regularly. Differences among means were verified using Cochran-Cox test. The results were shown in Table 1. In the study no 1 there were stated statistically significant lower values of LKM1 level in blood serum of patients as compared to controls ($p< 0.01$). Similarly, in the study no 2 statistically lower values of LKM1 level were observed in comparison with controls ($p< 0.001$).

No statistically significant differences of LKM1 level were stated in the study no 1 as compared to the study no 2 ($p>0.05$).

Table 1. LKM-1 serum level in patients with infectious mononucleosis (IM) and in healthy people

	Number of examined (n)	LKM1 serum level Mean (U/ml)	Standard deviation
Control group	20	1.42	0.57
Study no 1	52	0.76	0.33
Study no 2	52	0.62	0.31

In the performed studies in patients, being in severe symptomatic period of IM, there was stated the decrease of LKM1 level in blood serum which was maintained after regression of clinical symptoms.

DISCUSSION

LKM1, first described by R i z z e.t t o et al. in 1973, is known to recognize a 50-kDa protein in rat microsomes located primarily in the smooth endoplasmic reticulum and a 48-kDa protein in human liver microsomes. This protein in human liver was later identified as cytochrome P4502D6 (CYP2D6). The catalytic function of this cytochrome is inhibited *in vitro* by incubation with LKM1-positive serum (4). LKM1 was originally described as the serological hallmark of autoimmune hepatitis (AIH) type 2, and was later reported to be present in up to 10% of patients with chronic hepatitis C virus (HCV) infection (5). A basic fundamental requirement to hypothesise a role for any autoreactivity in the development and progression of immunologically mediated, organ specific damage is the physical accessibility of the target antigen(s) in the affected organ by the supposed effector mechanisms – that is, autoantibodies and autoreactive T cells. LKM1 is suggested to have direct liver damaging activity, but evidence on the presence of its target antigen on the hepatocyte plasma membrane – a prerequisite for antibody accessibility – is so far inconclusive.

The main LKM1 autoantigen cytochrome P450IID6 (CYP2D6) belongs to the P450 cytochrome family. CYP2D6, as well as other isoforms of cytochrome P450, are largely located in the hepatocyte endoplasmic reticulum. However, with this subcellular distribution, CYP2D6 would not be available as a target of a hypothetical liver specific autoimmune process. A growing body of evidence indicates that a massive flow of vesicles from the endoplasmatic reticulum through the Golgi apparatus to the plasma membrane transports endoluminal or membrane bound proteins, including cytochrome P450s, to the plasma membrane. Several isophorms of cytochrome P450, including CYP2D6, may be exposed on the outer surface of the hepatocyte plasma membrane. However, the presence of CYP2D6 on the hepatocyte plasma membrane is controversial, as studies using LKM1 positine autoimmune sera failed to show its presence on the hepatocyte surface (7).

It seems to be interesting that the presence of B cell epitopes on CYP2D6 has been investigated by several groups. EBV *in vitro* infection of B lymphocytes, resulting in activation and continuous proliferation of latently-infected cells as immortalized lymphoblastoid cell lines (LCL), is a highly efficient process. This remarkable event is brought about by the products of nine well characterized latent genes which are the first to be expressed in the infectious cycle: six Epstein-Barr viral nuclear antigens, EBNA-1, EBNA-2, EBNA-3A, EBNA-3B, EBNA-3C and leader protein (LP), and three latent membrane proteins, LMP-1, LMP-2A, LMP-2B. In addition, two untranslated RNAs (EBER) and a family of transcripts from the BamH1A region of the genom are expressed but their functions are unknown. The knowledge of the functions of the latent proteins is extensive but incomplete. EBNA-1, EBNA-2, EBNA-3A EBNA-3C and LMP are

essential for *in vitro* B-cell immortalization and under the pivotal influence of the transactivator protein EBNA-2, they act in concert to drive cells into continuous proliferation, whilst fixing them at the lymphoblastoid stage of differentiation and blocking progression to lytic replication in the majority of infected cells (2, 4). It was reported that hepatic involvement of CAEBV should be considered as differential diagnosis in cases showing liver dysfunction with clinical and biochemical features observed in AIH (1).

In our study, we observed statistically lower values of LKM-1 level in comparison with controls ($p<0.001$). In performed studies in patients, being in severe symptomatic period of IM, there was stated the decrease of LKM1 level in blood serum which was maintained after regression of clinical symptoms.

Autoimmune hepatitis (AIH) is a progressive inflammatory liver disease characterized by specific duration of symptoms (above 6 months), three- to tenfold elevation in serum aminotransferase activities, twofold elevation in gamma-globulin, high titers (above 1: 40) of circulating autoantibodies. Antinuclear antibodies (ANA), smooth muscle antibodies (SMA), and antibodies to liver/kidney microsome type 1 (anti-LKM1) are the commonly available markers of AIH. SMA and ANA lack disease specificity and they are present commonly in different liver diseases. Anti-LKM1 usually connote a type 2 form of AIH which involves children and responds to corticosteroids therapy. Other autoantibodies, such as those against actin (SMA-AA), the liver-cytosolic antigen (anti-LC1), the liver-specific membrane lipoprotein (anti-LPS), asialoglycoprotein receptor (anti-ASGPR), have greater disease specificity by less availability (6).

The question of LKM1 involvement in immunologically mediated liver damage therefore remains open.

REFERENCES

1. Chiba T. et al.: Fatal chronic active Epstein-Barr virus infection mimicking autoimmune hepatitis. Eur. J. Gastroenterol. Hepatol., 16, 2, 225, 2004.
2. Crawford D. H.: Biology and disease associations of Epstein-Barr virus. Phil. Trans. R. Soc. Lond., B, 356, 461, 2001.
3. Hinedi T. B., Koff R. S.: Cholestatic hepatitis induced by Epstein-Barr virus infection in an adult. Dig. Dis. Sci., 48, 3, 539, 2003.
4. Kerkar N. et al.: Cytochrome $P4502D6_{193-212}$: a new immunodominant epitope and target of virus/self cross-reactivity in liver/kidney microsomal autoantibody type 1-positive liver disease. J. Immunol., 170, 1481, 2003.
5. Ma Y. et. al.: Key residues of a major cytochrome P4502D6 epitope are located on the surface of the molecule. J. Immunol., 169, 277, 2002.
6. Marcinkiewicz M. et al.: Rola wybranych markerów serologicznych w diagnostyce autoimmunologicznego zapalenia wątroby. Przegl. Lek., 55, 11, 614, 1998.
7. Muratori L. et al.: Liver/kidney microsomal antibody type 1 targets CYP2D6 on hepatocyte plasma membrane. Gut, 46, 553, 2000.
8. Yuge A. et al.: Persistent hepatitis associated with chronic active Epstein-Barr virus infection. Pediatr. Infect. Dis. J., 23, 1, 74, 2004.

SUMMARY

The study comprised 72 people including 52 patients (29 women and 23 men), being in severe symptomatic period of IM and 20 healthy people (11 women and 9 men). The diagnosis of IM was confirmed by the presence of anti-VCA IgM antibodies in the quantity above 20 AU/ml (ELISA, Organon Technika). Levels of LKM1 in blood serum of patients were measured twice using Enzyme Immunoassay method (Pharmacia Upjohn): on the second day of hospitalisation (study no 1) and after regression of clinical symptoms (study no 2). Obtained data were statistically analysed

using Kolmogorow-Smirnov and Cochran-Cox methods. In the study no 1 there were statistically significant lower values of LKM1 level in blood serum of patients as compared to controls ($p< 0.01$). Similarly, in the study no 2 statistically lower values of LKM1 level were observed in comparison with controls ($p< 0.001$). No statistically significant differences of LKM1 level were stated in the study no 1 as compared to the study no 2 ($p>0.05$). In performed studies in patients, being in severe symptomatic period of IM, there was stated the decrease of LKM1 level in blood serum which was maintained after regression of clinical symptoms.

Dynamika poziomu LKM1 w surowicy krwi chorych
w ostrym objawowym okresie mononukleozy zakaźnej

Badaniami objęto 72 osoby, w tym 52 pacjentów (29 kobiet i 23 mężczyzn) w ostrym objawowym okresie mononukleozy zakaźnej oraz 20 osób zdrowych (11 kobiet i 9 mężczyzn). Diagnozę ustalono w oparciu o obraz kliniczny, badania laboratoryjne, potwierdzono obecnością przeciwciał anty-VCA IgM w mianie powyżej 20 AU/ml (ELISA, Organon Technika). Poziom LKM1 w surowicy krwi chorych oznaczono metodą Enzyme Immunoassay (Pharmacia Upjohn) dwukrotnie: w drugim dniu hospitalizacji (badanie 1) i po ustąpieniu objawów klinicznych (badanie 2). Uzyskane dane poddano analizie statystycznej z użyciem testów Kolmogorowa-Smirnowa i Cochrana-Coxa. W badaniu 1 stwierdzono statystycznie istotny spadek poziomu LKM1 w surowicy krwi chorych w porównaniu z kontrolą. Podobnie w badaniu 2 poziom LKM1 był statystycznie istotnie niższy w porównaniu z kontrolą. Nie stwierdzono statystycznie istotnych różnic w poziomie LKM1 w badaniu pierwszym w porównaniu z badaniem drugim. W przeprowadzonych badaniach w ostrym objawowym okresie mononukleozy zakaźnej oberwowano obniżenie poziomu LKM1 w surowicy krwi chorych w porównaniu z grupą osób zdrowych.