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Comparison of L-FABP, ALT and AST Levels in Chronic Hepatitis C

[Kronik Hepatit C'de L-FABP, ALT ve AST Düzeylerinin Karşılaştırılması]

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ABSTRACT

Purpose: To determine the plasma level of liver- type fatty acid binding protein (L-FABP) in chronic hepatitis C (CHC), and to compare this to Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) levels.

Methods: We tested 38 biopsy-proven CHC patients and 33 age- and sex- matched healthy controls in whom biopsy was not performed for ethical considerations. Patients with persistently elevated plasma aminotransferase levels (ALT and/or AST), and positive anti-HCV antibody and HCV-RNA results were included, but not those with liver cirrhosis, chronic alcohol consumption, severe and/or acute cardiac, renal, cerebrovascular or intestinal disease, seropositive for Hepatitis B virus or HIV. In addition to routine biochemical assessment, L-FABP was determined using a solid phase specific sandwich ELISA.

Results: Plasma ALT and AST level were significantly higher in the patient group than in the control group (57.7±27.9 IU/L vs 19.0± 7.7 IU/L, $p<0.01$ and 44.5±19.2 IU/L vs 18.3± 4.2 IU/L, $p<0.01$, respectively). Plasma L-FABP was significantly higher in the patient group than in the control group (5480.3± 4387.8 ng/mL vs 1710.5± 911.6 ng/mL, $p<0.01$). No correlation was found between L-FABP and either of the enzyme levels. ROC analysis produced cut-off values above which the likelihood of CHC increased. The cut-off value was 27.5 IU/L for ALT and 23.5 IU/L for AST. The cut-off value for L-FABP was 2600 ng/mL.

Conclusion: L-FABP is elevated significantly in CHC when compared to healthy controls, independent of aminotranferase levels. Further studies are warranted to evaluate the potential use of L-FABP in the setting of CHC.

Conflict of interest: The authors have no conflict of interest to declare.

Keywords: Chronic Hepatitis C, Hepatocellular Damage, Liver-Type Fatty Acid Binding Protein, AST, ALT

ÖZET

Amaç: Kronik Hepatit C hastalarında Karaciğer Tipi Yağ Asidi Bağlayıcı Protein (L-FABP) düzeylerinin belirlenmesi ve bu düzeylerin aspartat aminotransferaz (AST) ve alanin aminotransferaz (ALT) düzeyleri ile ilişkisinin tanımlanması amaçlanmıştır.

Metotlar: Tanısı biyopsiyle doğrulanmış 38 kronik Hepatit C hastası ve etik nedenlerden ötürü biyopsi yapılmamış olan 33 sağlıklı kontrol çalışmaya alınmıştır. Süregelen aminotransferaz (ALT ve/veya AST) yüksekliği, pozitif anti-HCV antikoru ve pozitif HCV-RNA sonucu diğer ön koşullardır. Karaciğer sirozu, kronik alkol kullanımı, ciddi ve/veya akut kardiyak, renal, serebral veya intestinal hastalığı olanlar, Hepatit B veya HIV için seropozitif hastalar çalışmaya dahil edilmemiştir. Hastaların rutin biyokimyasal değerlendirmelerinin yanı sıra, solid faz özgül sandviç ELISA yöntemi ile plazma L-FABP düzeyleri ölçülmüştür.

Bulgular: Kronik Hepatit C grubunda plazma ALT ve AST değerleri kontrol grubu ile karşılaştırıldığında anlamlı olarak daha yüksek bulunmuştur (sırasıyla 57.7±27.9 IU/L'e karşılık 19.0± 7.7 IU/L, $p<0.01$ ve 44.5±19.2 IU/L'e karşılık 18.3± 4.2 IU/L, $p<0.01$). Hasta grubunda plazma L-FABP düzeyleri kontrol grubuna göre anlamlı derecede yüksek bulunmuştur (5480.3± 4387.8' ng/mL'ye karşılık 1710.5± 911.6 ng/mL, $p<0.01$). L-FABP ve aminotransferaz düzeyleri arasında korelasyon olmadığı görülmüştür. ROC analizinde, aşıldığında kronik Hepatit C olasılığının arttığı kesim noktaları ALT için 27,5 IU/L, AST için 23,5 IU/L ve L-FABP için 2600,0 ng/mL olarak belirlenmiştir.

Sonuçlar: Sağlıklı kontrollerle karşılaştırıldığında, kronik Hepatit C hastalarında plazma L-FABP düzeyleri, karaciğer aminotransferaz düzeylerinden bağımsız olarak, anlamlı derecede yüksek bulunmuştur. Bulgularımız L-FABP'ın kronik Hepatit C hastalarında hepatosit hasarının bağımsız bir göstergesi olabileceğini düşündürmektedir. Bu hipotez geniş ölçekli çalışmalarla doğrulanmalıdır.

Anahtar Kelimeler: Kronik Hepatit C, Hepatosellüler hasar, Karaciğer Tipi Yağ Asidi Bağlayıcı Protein, AST, ALT

Introduction

Hepatitis C virus (HCV) is one of the most common causative agents of chronic viral hepatitis. Chronic hepatitis C (CHC) can progress to cirrhosis and eventually to hepatocellular carcinoma, and thus constitutes a major public health problem. Biochemical markers of hepatocellular injury, especially the liver aminotransferases, have a very important role in the diagnosis, follow-up, and risk stratification of CHC [1-4].

Release of cytoplasmic proteins from damaged hepatocytes into the vascular system occurs as a result of viral or toxic hepatitis, ischemia or congestion of the liver, shock, trauma, or rejection after transplantation. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are routinely employed for the initial assessment and monitoring of hepatic disease [3]. Currently, ALT is the only marker with adequate specificity for liver disease, but its rather large size (96 kDa) may retard the rise of its concentration in the plasma, thus rendering its use in detection of early stages of liver damage [3]. Alpha glutathione S-transferase (α-GST), which is normally present in liver, kidney and intestine, has been shown to be a useful marker for detection of hepatocellular injury due to rejection after liver transplantation [5].

Markers that can be detected earlier and that are more sensitive than ALT are needed [3]. Fatty acid-binding proteins (FABPs) are a family of intracellular proteins of molecular mass 15 kDa that function in the intracellular transport of long-chain fatty acids. Nine FABPs have been identified of which liver-type (L-FABP) is mainly present in mammalian hepatocytes and enterocytes. It constitutes 2–5% of cytosolic protein content, and very rapidly leaks out of damaged cells, leading to an early rise in plasma level [6-9]. L-FABP has effective endogenous protectant, including antioxidant, properties [10-12]. L-FABP binds long-chain fatty acids and other ligands such as fibrates [13] and endogenous PPARα [14]. It rises significantly after bile duct ligation in rats [15], and during episodes of acute rejection of liver transplant [16].

This study was designed to compare plasma L-FABP, AST and ALT levels in CHC patients in view of no published data concerning L-FABP levels in chronic Hepatitis C infection.

Materials and Methods

We enrolled 38 biopsy-proven CHC patients (14 males and 24 females) referred to the Gazi University Gastroenterology Department and 33 age- and sex- matched healthy controls (15 males and 18 females). All of the patients were newly diagnosed with CHC. The control group consisted of individuals without any systemic disease whose biochemical, hematological, virological serum markers (HbsAg, anti-HBc total, anti-HCV, antiHIV) and abdominal ultrasonographies were normal. Liver biopsy was not performed in the control group for ethical considerations. Informed consent was obtained from all subjects. The study protocol was approved by the local ethics committee.

The patient group included those who showed persistently elevated serum transaminase levels for 6 months, who were positive for both anti-HCV antibody and HCV-RNA, and had liver biopsy results compatible with CHC.

Excluded were patients with liver cirrhosis, those in whom liver biopsy was contraindicated, with positive serological markers for hepatitis B virus or for anti-HIV antibody, with chronic alcohol consumption in excess of 20 g per day, with signs of autoimmune or metabolic liver disease, with severe cardiac disease, with acute renal damage or failure, with acute cerebrovascular disease, with skeletal muscle injury or myositis, or with any acute intestinal condition.

Plasma samples were assayed for AST and ALT as well as gamma-glutamyl transferase (GGT), bilirubin, and L-FABP levels.

AST was determined using a standard UV absorption technique [17]. L-FABP levels were determined using a specific sandwich ELISA method (Hycult Biotechnology B.V., Uden, Netherlands).

Liver biopsies were performed and all were evaluated by the same pathologist using the Metavir scoring system [18].

Serum HCV-RNA level was determined by reverse transcriptase – PCR using a commercial kit (MagAttract Virus Mini M48 Kit, *RealTime*™ HCV Amplification Reagent Kit, Abbott) and Anti-HCV antibody was determined by ELISA (chemiluminescence).

Genotype analysis of all subjects was performed by the Line Assay (innolipa) strip method and all were determined as genotype 1b [19].

Results were evaluated using the SPSS 12.0 software (SPSS Inc., Chicago,Illinois, USA). Comparisons were made using the Mann-Whitney *U* test between two groups [20]. The Pearson Chi-square test [21] and Fisher's exact test [22] were used for categorical data. ROC analysis was performed for AST, ALT, and L-FABP; area under curve and confidence interval for each of these parameters were calculated. Cut-off values determining the likelihood of CHC were also determined. The relation between variables was examined with correlation analysis. P values of less than 0.05 were considered to be statistically significant.

Results

Mean age at enrollment was 51.1± 11.1 years in the CHC group and 49.8± 12.8 years in the control group (p=0.556) (Table 1). The distribution of individuals according to body mass index (BMI) did not significantly differ between the CHC and control groups (p=0.173) (Table 2).

Plasma ALT and AST levels were significantly higher in the CHC group than in the control group (57.7±27.9 IU/L vs 19.0± 7.7 IU/L, p<0.01 and 44.5±19.2 IU/L vs 18.3± 4.2 IU/L, p<0.01, respectively). The levels of GGT were higher in the CHC group (48.3±34.3 vs 20.3±9.0, p<0.01). Total and direct bilirubin levels were significantly higher in the CHC group (see Table 3).

Table 1. Age and gender characteristics of CHC and control groups

	CHC	Control
Male, n (%)	14 (36,8)	15 (45,5)
Female, n (%)	24 (63,2)	18 (54,5)
Age, Mean ± SD	51.1 ± 11.0	49.8 ± 12.8

Numbers in brackets denote percentages
CHC: Chronic Hepatitis C

Table 2. The distribution of individuals according to Body Mass Index in CHC and control groups

	CHC		Control	
	n	%	n	%
Normal[1]	13	34,2	14	42,4
Overweight[2]	15	39,5	16	48,5
Obese[3]	10	26,3	3	9,1

CHC: Chronic Hepatitis C
SD: Standard Deviation
[1]Body Mass Index between 18.5 and 24.9 kg/m^2
[2]Body Mass Index between 25 and 29.9 kg/m^2
[3]Body Mass Index equal to or greater than 30 kg/m^2

Table 3. Biochemical findings

		N	Mean	Median	SD	Min	Max	P
ALT	CHC	38	57,7	51,5	27,9	16	157	**P=0,0001**
	Control	33	19,0	17	7,7	8	43	
AST	CHC	38	44,5	42	19,2	19	102	**P=0,0001**
	Control	33	18,3	18	4,2	6	25	
GGT	CHC	38	48,3	37,5	34,3	10	150	**P=0,0001**
	Control	33	20,3	18	9,0	9	50	
T. bil	CHC	38	0,8	0,645	0,6	0,19	3,31	P=0,053
	Control	33	0,6	0,53	0,2	0,28	1,38	
D. bil	CHC	38	0,3	0,285	0,2	0,1	0,93	**P=0,055**
	Control	33	0,2	0,21	0,1	0,1	0,46	
L-FABP	CHC	38	5480,3	5000	4387,8	1700	25600	**P=0,0001**
	Control	33	1710,5	1500	911,6	640	4000	

*p<0.05, Statistically Significant
CHC: Chronic Hepatitis C, ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase, GGT: Gamma-Glutamyl Transferase, T. bil: Total bilirubin, D. bil: Direct bilirubin , L-FABP: Liver Type Fatty Acid Binding Protein

Plasma level of L-FABP was significantly higher in the CHC group than in the control group (5480.3± 4387.8 ng/mL vs 1710.5± 911.6 ng/mL, p< 0.01). We did not observe any correlation between L-FABP levels and neither aminotransferase levels (Figure 1).

L-FABP/ALT ratio was not significantly different in the CHC and control groups (94.97 vs 90.02, p=NS).

Subgroup analysis of patients with normal (≤40 IU/mL) against those with elevated (≥40 IU/mL) ALT levels showed that the L-FABP levels were not significantly different. However, L-FABP level in patients with normal ALT level tended to be higher than that in controls, but the difference did not reach statistical significance.

ROC analysis for ALT, AST, and L-FABP was performed. None of these parameters showed any superiority to the others in terms of area under curve. It was noted that the area under curve approximated the numerical value 1 for all three parameters studied in ROC analysis (Table 4). ROC curves for ALT, AST, and L-FABP are given in Figure 2.

ROC analysis also produced cut-off values above which the likelihood of CHC increased. The cut-off value was 27.5 IU/L for ALT and 23,5 IU/L for AST. The cut-off value for L-FABP was 2600 ng/mL.

Discussion

CHC is a serious condition that leads to debilitating liver cirrhosis and fatal hepatocellular carcinoma. Sensitive detection of hepatocellular injury can aid in diagnosis and in monitoring of CHC. Of the markers routinely used for these purposes, only ALT has adequate sensitivity but rises slowly. Thus, a specific marker the levels of which rise rapidly after hepatocellular damage would be ideal.

L-FABP, owing to its small molecular weight (15 kDa), leaks out of damaged hepatocytes very early and rapidly following injury. L-FABP levels rise earlier than the levels of ALT, making earlier detection of acute liver transplant rejection possible. The high intracellular he-

Table 4. ROC Analysis Statistics

Test Result Variable	Area Under Curve	p	Asymptotic 95% Confidence Interval	
			Upper Bound	Lower Bound
ALT	0,954	0,000	0,910	0,998
AST	0,967	0,000	0,931	1,002
L-FABP	0,934	0,000	0,882	0,986

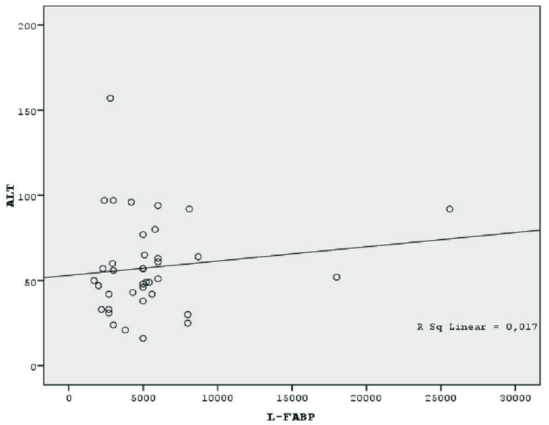

Figure 1. The correlation graphic of ALT and L-FABP. No correlation was observed between ALT and L-FABP.

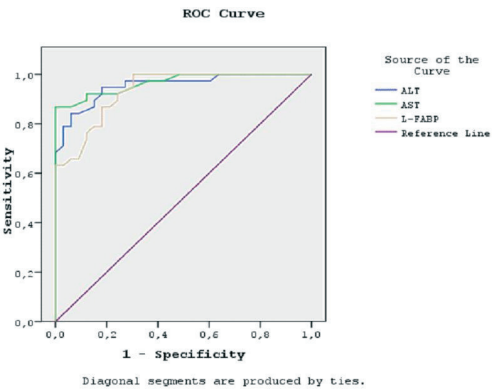

Figure 2. ROC Curves for ALT, AST, and L-FABP.

patic concentration of L-FABP increases its sensitivity for hepatocellular damage. L-FABP is normally a cytoplasmic protein and hence its serum levels are neglectably low. For the sandwich ELISA method, which is used for the detection of L-FABP, the detection limit of the assay is 0.1 ng/mL, and the intra- and interassay CVs are <5% and <15%, respectively.

The fact that L-FABP has cysteine and methionine groups implies that this protein may also have antioxidant properties. In an *in vitro* experimental model of hepatic oxidative stress, it was demonstrated that L-FABP acts as an endogenous cytoprotectant [10]. In our study, we found elevated plasma levels of L-FABP as a result of leakage from damaged hepatocytes in CHC. Considering that oxidative stress is a major mechanism of hepatocellular damage in CHC, the role of cytoplasmic L-FABP against oxidative stress remains to be elucidated in this clinical setting.

To the best of our knowledge, this is the first report on the plasma levels of L-FABP in CHC. We also determined a cut-off value for L-FABP above which the likelihood of CHC increases. This cut-off value, which is 2600 ng/mL, may be valuable in aiding the diagnosis and risk stratification of CHC. In ROC analysis, L-FABP was not superior to AST or ALT in terms of area under curve. However, the knowledge from previous studies that its levels raise earlier than those of AST and ALT may confer an advantage on L-FABP.

The main limitations of L-FABP were that our sample size was small and lacking a follow-up, so we could not observe changes of L-FABP levels over time in the patient and control groups.

ALT level may be normal in CHC and marked fibrosis and even cirrhosis can be encountered in patients with such levels [23-25]. This may explain why we found no correlation between ALT and L-FABP levels. However, L-FABP level may reflect hepatocellular damage in presence of normal ALT levels.

Our results suggest that L-FABP may be a sensitive marker of hepatocellular damage in the setting of CHC. This needs to be tested in larger studies. This sensitive marker of hepatocyte injury may potentially be useful for the detection of hepatocyte damage, possibly including flare ups in the course of CHC.

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References

[1] Lee MH, Yang HI, Lu SN, Jen CL, Yeh SH, Liu CJ, Chen PJ, You SL, Wang LY, Chen WJ, Chen CJ. (2010) Hepatitis C virus seromarkers and subsequent risk of hepatocellular carcinoma: long term predictors from a community-based cohort study. J Clin Oncol. 28(30): 4587-4593.

[2] Liu CH, Liang CC, Liu CJ, Hsu SJ, Lin JW, Chen SI, Hung PH, Tsai HB, Lai MY, Chen PJ, Chen JH, Chen DS, Kao JH. (2010) The ratio of aminotransferaseto platelets is a useful index for predicting hepatic fibrosis in hemodialysis patients with chronic hepatitis C. Kidney Int. 78(1):103-109.

[3] Dufour DR, Lott JA, Nolte FS, Gretch DR, Koff RS, Seeff LB. (2000) Diagnosis and monitoring of hepatic injury: I. Performance characteristics of laboratory tests. Clin Chem. 46(12):2027–2049.

[4] Trull AK. (2001) The clinical validation of novel strategies for monitoring transplant recipients. Clin Biochem. 34:3–7.

[5] Hughes VF, Trull AK, Gimson A, Friend PJ, Jamieson N, Duncan A, *et al*. (1997) Randomized trial to evaluate the clinical benefits of serum alpha-glutathione *S*-transferase concentration monitoring after liver transplantation. Transplantation. 64:1446–1452.

[6] Glatz JF, van der Vusse GJ. (1996) Cellular fatty acid-binding proteins: their function and physiological significance. Prog Lipid Res. 35:243–282.

[7] Bass NM, Barker ME, Manning JA, Jones AL, Ockner RK. (1989) Acinar heterogeneity of fatty acid-binding protein expression in the livers of male, female and clofibrate-treated rats. Hepatology 9:12–21.

[8] Ockner RK, Manning JA, Kane JP. (1982). Fatty acid-binding protein. Isolation from rat liver, characterization, and immunochemical quantification. Biol Chem. 257:7872–7878.

[9] Gordon JI, Alpers DH, Ockner RK, Strauss AW. (1983) The nucleotide sequence of rat liver fatty acid-binding protein mRNA. J Biol Chem. 258:3356–3363.

[10] Rajaraman G, Wang GQ, Yan J, Jiang P, Gong Y, Burczynski FJ. (2007) Role of cytosolic liver fatty acid binding protein in hepatocellular oxidative stress: effect of dexamethasone and clofibrate treatment. Mol Cell Biochem. 295(1-2):27-34.

[11] Wang G, Gong Y, Anderson J, Sun D, Minuk G, Roberts MS, Burczynski FJ. (2005) Antioxidative function of L-FABP in L-FABP stably transfected Chang liver cells. Hepatology. 42(4):871-879.

[12] Liu Y, Wang J, Wei Y, Zhang H, Xu M, Dai J. (2008) Induction of time-dependent oxidative stress and related transcriptional effects of perfluorododecanoic acid in zebrafish liver. Aquat Toxicol. 89(4):242-250.

[13] Chuang S, Velkov T, Horne J, Wielens J, Chalmers DK, Porter CJ, Scanlon MJ. (2009) Probing the fibrate binding specifity of rat liver fatty acid binding protein. J Med Chem. 52(17):5344-5355.

[14] Hostetler HA, McIntosh AL, Atshaves BP, Storey SM, Payne HR, Kier AB, Schroeder F. (2009) L-FABP directly interacts with PPARα in cultured primary hepatocytes. J Lipid Res. 50(8):1663-1675.

[15] Wang G, Shen H, Rajaraman G, Roberts MS, Gong Y, Jiang P, Burczynski F. (2007) Expression and antioxidant function of liver fatty acid binding protein in normal and bile-duct ligated rats. Eur J Pharmacol. 560(1):61-68.

[16] Pelsers MAL, Morovat A, Alexander GJM, Hermens WT, Trull AK, Glatz JFC. (2002) Liver Fatty Acid Binding Protein as a Sensitive Serum Marker of Acute Hepatocellular Damage in Liver Transplant Recipients. Clinical Chemistry 48:2055-2057.

[17] Rej R. (1984) Measurement of aminotransferases: Part 1. Aspartate aminotransferase. Crit Rev Clin Lab Sci.21 (2):99-186.

[18] Bedossa P, Poynard T (1996) An algorithm for the grading of activity in Chronic Hepatitis C. Hepatology. 24:289-293.

[19] Bouchardeau F, Cantaloube JF, Chevaliez S, Portal C, Razer A, Lefrere JJ, Pawlotsky JM, De Micco P, Laperche S. (2007) Improvement of Hepatitis C Virus (HCV) genotype determination with the new version of the INNO-LIPA HCV assay. Journal of Clinical Microbiology. 45:1140-1145.

[20] Mann HB, Whitney DR. (1947) On a test of whether one of two random variables is stochastically larger than the other. The Annals of Mathematical Statistics.18 (1):50-60.

[21] Pearson K. (1950) On the criterion that a given system of deviations from the probable in the case of a correlated system of variables is such that it can be reasonably supposed to have arisen from random sampling. Philosophical Magazine.302:157-175.

[22] Fisher R.A. (1922) On the interpretation of $\chi 2$ from contingency tables, and the calculation of P. Journal of the Royal Statistical Society.85 (1):87-94.

[23] Puoti C, Castellacci R, Montagnese F. (2000) Hepatitis C virus carriers with persistently normal aminotransferase levels: healthy people or true patients? Dig Liver Dis.32:634-643.

[24] Puoti C, Guido M, Mangia A, Persico M, Prati D. (2003) Clinical management of HCV carriers with normal aminotransferase levels. Dig Liver Dis. 35:362-369.

[25] Puoti C, Magrini A, Stati T, Rigato P, Montagnese F, Rossi P, et al (1997) Clinical, histological, and virological features of hepatitis C virus carriers with persistently normal or abnormal alanine transaminase levels. Hepatology. 26:1393-1398.