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Alpha-1 antitrypsin deficiency in patients with chronic hepatitis

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Background/aims: *Alpha-1 antitrypsin deficiency causes accumulation of mutant alpha-1 antitrypsin molecules in hepatocytes, and is attributed to severe liver injury even in heterozygous state. However, there is a question as to whether alpha-1 antitrypsin deficiency is only a cause of liver injury or has a worsening effect on the underlying liver disease. We aimed to determine the role of alpha-1 antitrypsin deficiency in the ongoing chronic hepatitic process.* ***Materials and Methods:*** *Fifty-four patients with the diagnosis of chronic hepatitis by liver biopsy (36 chronic hepatitis B virus, 8 chronic hepatitis C virus, 7 non-alcoholic steatohepatitis, 2 primary biliary cirrhosis, and 1 autoimmune hepatitis) and 51 age- and sex-matched control subjects chosen from among healthy blood donors were included in the study. Isoelectric focusing for identifying alpha-1 antitrypsin phenotypes was performed in all patients and control subjects, whereas the histopathological examination was done only in patients.* ***Results:*** *Alpha-1 antitrypsin-deficient variant was absent in patients and controls. The mean serum alpha-1 antitrypsin level was significantly lower in patients (157.4±33 mg/dl) than controls (134.8±30 mg/dl) (p<0.00). Histological activity index and fibrosis grade in the liver were not related to the serum alpha-1 antitrypsin level (p: 0.276 and 0.902, respectively). Additionally, the serum alpha-1 antitrypsin levels among normal variants of alpha-1 antitrypsin did not differ according to the underlying liver diseases (p: 0.928).* ***Conclusions:*** *This prospective case-control study could not define any additional effect of alpha-1 antitrypsin deficiency on liver histopathology in chronic hepatitis patients.*

Key words: *Alpha-1 antitrypsin deficiency, chronic hepatitis, antitrypsin phenotypes*

Kronik karaciğer hastalığında alfa-1 antitripsin eksikliği

Amaç: *Alfa-1 antitripsin eksikliği, mutant alfa-1 antitripsin molekülünün hepatositte depolanması sonucu karaciğer hasarına sebep olmaktadır. Alfa-1 antitripsin eksikliğinin karaciğer hasarının oluşması için tek başına yeterli olup olmadığı bilinmemektedir. Bu çalışmada, kronik hepatit tanısı olan hastalardaki alfa-1 antitripsin eksikliği varlığını tespit etmeyi amaçladık.* ***Gereç ve Yöntem:*** *Çalışmaya tanısı karaciğer biyopsisi ile konan 54 hasta (36 kronik hepatit B, 8 kronik hepatit C, 7 non-alkolik steatohepatit, 2 primer biliyer siroz ve 1 otoimmün hepatit) ve 51 yaş ve cinsiyet uyumlu gönüllü kan donörü dahil edildi. Bütün hastalarda ve kontrol grubunda alfa-1 antitripsin fenotiplendirmesi için isoelektrik fokuslama yöntemi kullanılırken hasta grubunda karaciğer biyopsi örneğinde histopatolojik inceleme yapıldı.* ***Bulgular:*** *Hiçbir hastada ve kontrol grubunda alfa-1 antitripsin eksik varyantı tespit edilmedi. Ortalama alfa-1 antitripsin serum düzeyi hasta grubunda kontrol grubuna göre anlamlı olarak daha düşük idi (sırasıyla 157.4±33 mg/dL ve 134.8±30 mg/dL, p<0.00). Karaciğer doku örneğindeki histopatolojik aktivite indeksi ve fibrosis evrelemesi ile alfa-1 antitripsin serum düzeyi arasında bir ilişki bulunmadı (sırasıyla, p:0.276 ve 0.902). Hasta grubundaki serum alfa-1 antitripsin düzeyi, altta yatan hastalığa gore bir farklılık göstermemektedir (p: 0. 928).* ***Sonuç:*** *Bu prospektif-vaka kontrol çalışmasında, alfa-1 antitripsin eksikliğinin kronik karaciğer hastalığı sürecine ek bir katkısı olup olmadığı değerlendirilememiştir.*

Anahtar kelimeler: Alfa-1 antitripsin eksikliği, kronik karaciğer hastalığı, antitripsin fenotiplendirmesi

INTRODUCTION

Alpha-1 antitrypsin (AT) is a glycoprotein produced mainly by hepatocytes (1). The accumulation of mutant AT molecules in the endoplasmic reticulum of hepatocytes is the main cause of liver in-

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jury in patients with AT deficiency (2). The clinical manifestations of AT deficiency have varied from severe lung and liver diseases to asymptomatic carrier state (3). Emphysema, chronic obstructive pulmonary disease, cirrhosis, and hepatocellular carcinoma are the severe diseases frequently attributed to AT deficiency. The AT gene, located on the long arm of chromosome 14, has approximately 100 alleles (5). PI M is the normal allele expressing the normal AT level in serum (150-350 mg/dl), whereas the PI S and PI Z are the deficient alleles expressing lower level of AT (100-200 mg/dl and 15-50 mg/dl, respectively) (5). The severity of the disease is mostly based on the state of the AT mutation, whether homozygous or heterozygous (4).

The cut-off point to estimate AT deficiency in liver diseases is controversial (6). A dilemma is also ongoing about the additional effect of c.-1973T >C polymorphism in getting liver disease rather than lung disease (7). In childhood, 14-16% of the patients undergoing liver transplantation due to liver failure are AT-deficient (8,9). The frequency AT deficiency was 7.3-8.2% in adults undergoing liver transplantation compared with 2.8% in the healthy population of the same country (10,11). AT deficiency is a determining factor for the age at which liver diseases/failure become symptomatic, such as the age of 58 years for ZZ variant versus 73 years for MZ variant. PI MZ was shown to be more in hepatitis C virus (HCV) and non-alcoholic steatohepatitis (NASH)-related cirrhosis than in healthy subjects (13). A study revealed that among patients with chronic HCV and non-alcoholic liver diseases, there was a relation between AT deficiency and the severity of the underlying liver diseases (14). However, an association between AT deficiency and chronic HCV could not be determined in a study of 1048 chronic HCV patients (15).

The aim of the present study was to determine whether AT deficiency has an additional role in ongoing fibrosis in Turkish patients with chronic liver diseases diagnosed by liver biopsies.

MATERIALS AND METHODS

Patients

The patients who underwent liver biopsy from January 2008 to December 2008 in the Gastroenterology Unit of Hacettepe Medical Faculty Hospital were included in the study after the approval of informed consent. Fifty-four patients with the diagnosis of chronic liver disease by liver biopsy were included in the study. None of them had clinical cirrhosis. Liver biopsies performed intercostally were processed for the presence of diastase-resistant, periodic acid-Schiff (PAS)-positive intracytoplasmic deposits as well as routine histological examination by a single pathologist. The routine histopathological examination in paraffin-embedded blocks was done after staining by hematoxylin-eosin stain. The histological activity index (HAI) used to evaluate the degree of inflammation in the liver based on the Knodell scoring system is scored from 0 to 18 (16). Liver fibrosis scores were assessed via the METAVIR scoring system. According to this scoring system, means of scores were as follows: F0, no fibrosis; F1, portal fibrosis without any septa; F2, portal fibrosis with rare septa; F3, numerous septa, but without cirrhosis; and F4, cirrhosis (17). The control group consisted of 51 age- and sex-matched healthy blood donors. Blood samples for AT quantitation and phenotyping were drawn before the biopsy procedure. Serum was separated from blood by centrifugation at 4°C and stored at -70°C until AT quantitative determination or AT phenotyping.

Quantitative Determination of AT Concentration

The serum AT level was measured by the rate immune nephelometric method (Immage® Immunochemistry System, Beckman Coulter, Fullerton, CA, USA) according to the manufacturer's instructions. The normal range for AT in serum samples was 88-174 mg/dl.

AT Phenotyping

Phenotypes were determined by isoelectric focusing (IEF) (Phastsystem®, automated electrophoresis, GE Healthcare BioSciences AB, Uppsala, Sweden). The apparatus comprises a separation and control unit for electrophoresis and an automated development unit for staining and destaining, which can be programmed and operated independently. Dehydrated polyacrylamide gels (PhastGel Dry IEF) were rehydrated by floating onto the rehydration solution containing Pharmalyte 4.2-4.9. All serum samples were treated with cysteine (90 mqL of serum with 10 mqL cysteine solution, 0.3 mol/L, pH 7.4) (18) and incubated overnight at 4°C. Cysteine reduced 0.8 mqL of serum samples was applied to rehydrated polyacrylamide IEF gels. IEF running was carried out according to the manufacturer's recommended

running conditions (19). Immediately after focusing, gels were fixed and stained with Phast Gel Blue R for Coomassie Blue staining with the automated development unit (19). When the intensity of the stained protein bands was poor, SilverSNAP stain kit II (Pierce, IL, USA) for silver staining was performed according to the manufacturer's recommendations. During each experiment, control serum specimens obtained from Dr. Diane W. Cox (University of Alberta, Edmonton, Alberta, Canada), normal PI MM or abnormal phenotypes, were run simultaneously for comparison (20).

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) ver. 13.0 was used for statistical analysis. Demographic data were shown as mean +/- SD. Chi-square test, Mann-Whitney U test and Kruskal-Wallis test were used to compare the groups. A p-value of <0.05 was considered as significant.

RESULTS

The age distribution of the patients was 18-65 years. The mean age was 40±12 years, which was similar between the patient and control groups (Table 1). The etiologies of liver diseases in the study group were as follows: 36 chronic HBV, 8 chronic HCV, 7 NASH, 2 primary biliary cirrhosis, and 1 autoimmune hepatitis.

All of the patients and controls had normal variant of AT (PI MM).The mean serum level of AT did not differ according to gender (p: 0.341 and 0.453) or the etiology of the liver diseases (Table 2). Serum AT levels were higher in patients than controls (p<0.00) (Table 2). There was no significant relationship between serum AT level and HAI or fibrosis (p: 0.276 and 0.902, respectively).

The median HAI was higher in chronic HBV patients than in chronic HCV patients, whereas the fibrosis grade in chronic HCV patients was higher (Table 1). None of the examinations was positive for the presence of diastase-resistant, PAS-positive intracytoplasmic deposits in the liver.

DISCUSSION

Although alpha-1 antitrypsin (AT) deficiency is not rare, it is an underdiagnosed hereditary disease leading to liver injury (21). As reported previously, there could be an association between AT deficiency and chronic liver disease (22). However, the exact role of AT deficiency in liver injury is still unclear. Rates of AT deficiency in child and adult patients undergoing liver transplantation were 14-16% (overall AT deficiencies), and 7.3-8.2% of the subjects were PI MZ (8-11). Despite these high percentages, the PI ZZ and PI SZ subjects who were diagnosed by routine screening in their childhood were healthy in their follow-up at the ages of 26 and 30 years (23,24). In the current study, we investigated the relation between AT deficiency and chronic liver diseases (36 chronic HBV, 8 chronic HCV, and 7 NASH). Since all of our patients and controls were wild type (PI MM), no relation between the AT-deficient variants and liver diseases could be detected. While the prevalences of PI MZ and PI ZZ were 1 per 143 and 1 per 10,000 in healthy Turkish blood donors (unpublished data) and AT-deficient alleles are estimated to be higher in chronic hepatitis patients, we enrolled 54 subjects in our patient group.

In a retrospective analysis of PIZ deposits in 1847 liver biopsies and 1030 autopsies, it was noted that 3.4% of biopsies and 1.8% of autopsies were positive for PIZ deposits, and all of them were PI MZ (25). In addition, the inflammatory activity and fibrosis score were found to be related with AT deficiency (21). However, decompensated liver diseases due to NASH and chronic HCV were repor-

Table 1. Demographic characteristics of the patient and control groups

	Patient Group N: 54	Control Group N: 51	P value
Mean age ± SD	40±12	38±13	0.308
Sex, Male/Female (%)	19/35 (35/65)	20/31 (39/61)	0.334
Mean serum level of AT (mg/dl)	157.4±33	134.8±30	<0.00
HAI in HBV, median (range)	5 (2-9)	NA	NA
Fibrosis in HBV, median (range)	2 (0-5)	NA	NA
Fibrosis in HCV, median (range)	2 (1-5)	NA	NA
HAI in HCV, median (range)	7 (4-11)	NA	NA

NA: Non-applicable. AT: Alpha-1 antitrypsin. HAI: Histological activity index. HBV: Hepatitis B virus. HCV: Hepatitis C virus.

Table 2. The mean serum levels of AT according to liver disease etiologies

	AT levels in serum (mg/dl)	P value
HBV (n: 36)	159.8±29.9	
HCV (n: 8)	165.0±20.0	0. 928
NASH (n: 7)	153.7±41.1	

HBV: Hepatitis B virus. HCV: Hepatitis C virus. NASH: Non-alcoholic steatohepatitis.

ted to be aggravated by AT deficiency (11). The percentages of AT deficiency in compensated and decompensated cirrhosis were 1.2% and 5.0% in chronic HCV patients and 2.8% and 7.3% in NASH patients, respectively (p: 0.004 and 0.017, respectively).

The earlier studies noted that PI MZ is more prevalent in cirrhosis than in healthy subjects (10,11,13). While none of the patients in our study was cirrhotic, whether the serum level of AT is related with HAI or fibrosis in the absence of AT deficiency was investigated. HAI and fibrosis grade were found to be independent of serum AT level in the patients with chronic hepatitis (p: 0.276 for HAI and 0.902 for fibrosis grade).

The cut-off level of serum AT for investigating AT deficiency in the case of suspected liver disease has been debated. When the cut-off was set as <100 mg/dl, failure to diagnose occurred in just 6.7% of cases (6). However, in the present study, the patient group had higher serum AT levels than the control group (157.4±33 mg/dl and 134.8±30 mg/dl, p<0.00). This can be easily attributed to the acute phase reactant effect of the AT molecule.

Our limitation is the low number of the patients included in the study. To ascertain the impact of AT deficiency on the underlying chronic hepatitic process and whether or not AT deficiency is a *de novo* cause of chronic hepatitis, we should reach approximately 1,000 patients in a study held in our country (unpublished data).

In conclusion, AT deficiency was not more frequent in patients with chronic hepatitis. As an acute phase reactant protein, serum AT level was increased in patients, although there was no correlation with HAI or fibrosis grade. It can be suggested that an additional effect of AT deficiency in chronic liver diseases resulted in higher inflammation. Its effect on fibrosis is still unclear and the effect of AT deficiency in liver disease is minimal, especially in low frequency areas of AT deficiency.

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