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A new risk factor for the development of non-alcoholic fatty liver disease: HLA complex genes

Mehmet ÇELİKBİLEK[1], Haldun SELÇUK[2], Uğur YILMAZ[2]

Departments of [1]Internal Medicine and [2]Gastroenterology, Başkent University School of Medicine, Ankara

Background/aims: *Several studies have emphasized the role of genetic factors in susceptibility to non-alcoholic fatty liver disease. The aim of this study was to examine the possible influence of human leukocyte antigen in the development of non-alcoholic fatty liver disease.* ***Methods:*** *Between January 2000 and January 2008, data of 655 donor candidates were examined from routinely performed abdominal ultrasonography and for aspartate aminotransferase, alanine aminotransferase, hepatitis B virus, hepatitis C virus, cytomegalovirus, human immunodeficiency virus, hepatic functions, and human leukocyte antigen class I and II antigens; data of 116 healthy candidates were also included in this study. To reduce the influence of possible confounding factors, we excluded diseases known to be associated with non-alcoholic fatty liver disease like obesity, diabetes mellitus, coronary artery disease, hyperlipidemia, and metabolic syndrome. Non-alcoholic fatty liver disease was diagnosed in 66 individuals (33 male, median age: 53.8 [range, 32-77 years]) by means of ultrasonography data, and 50 individuals, whose ultrasonography data did not show hepatosteatosis, comprised the control group (20 male, median age: 44.6 [range, 26-71 years]).* ***Results:*** *Human leukocyte antigen-B65 (28.8% vs 0%, p<0.001) and DQ5 (40.7% vs 16.1%, p<0.05) were found to be expressed significantly more in non-alcoholic fatty liver disease compared with controls. Serum alanine aminotransferase (27.1 IU/L vs 20 IU/L, p<0.05) was significantly higher in the study group.* ***Conclusions:*** *Our preliminary study suggests that human leukocyte antigen plays a role in the pathogenesis of non-alcoholic fatty liver disease; however, more studies are needed to clarify these data.*

Key words: Non-alcoholic fatty liver disease, genetic, hepatosteatosis, human leukocyte antigen (HLA)

Non alkolik yağlı karaciğer hastalığının gelişiminde yeni bir risk faktörü: HLA kompleks genleri

Amaç: *Çalışmalar genetik faktörlerin non alkolik yağlı karaciğer hastalığına yatkınlıkta etkili olduğunu göstermektedir. Bizim çalışmamızda non alkolik yağlı karaciğer hastalığı gelişiminde insan lökosit antijenlerinin muhtemel etkilerinin araştırılması amaçlanmıştır.* ***Yöntem:*** *Çalışmada ocak 2000 ve ocak 2008 tarihleri arasında hastanemize böbrek nakil verici adayı olarak başvuran kişilerin verileri kullanılmıştır. Her böbrek nakil verici adayına batın ultrasononografisi, HLA sınıf 1ve 2 antijenleri, karaciğer fonksiyon testleri, viral testler rutin olarak yapılmaktadır. Çalışmada 116 sağlıklı verici adayının bilgileri kullanılmıştır. Muhtemel genetik etkiyi engellemek için obezite, diyabetes mellitus, koroner arter hastalığı, hiperlipidemi ve metabolik sendrom gibi non alkolik yağlı karaciğer hastalığı ile yakın ilişki gösteren hastalıklar dışlanmıştır. Ultrasonografi ile 66 vericide non alkolik yağlı karaciğer hastalığı saptandı (33 erkek, ortalama yaş: 53,8 [yaş aralığı, 32-77]) ve ultrasonografisi normal 50 verici adayı kontrol grubu olarak alındı (20 erkek, ortalama yaş: 44,6 [yaş aralığı, 26-71]).* ***Bulgular:*** *Kontrol grubuyla karşılaştırıldığında non alkolik yağlı karaciğer hastalığı grubunda HLA B65 (28.8% vs 0%, p<0.001) ve DQ5 (40.7% vs 16.1%, p<0.05) istatistiksel olarak anlamlı yüksek bulunmuştur. Serum alanin aminotransferaz (27.1 ıu/l vs 20 ıu/l, p<0.05) non alkolik yağlı karaciğer hastalığı saptanan grupta anlamlı yüksek bulunmuştur.* ***Sonuç:*** *Ön çalışma niteliğinde olan bu çalışmada insan lökosit anitjenlerinin non alkolik yağlı karaciğer hastalığı patogenezinde rol alabileceği saptanılmıştır. Bu veriyi doğrulamak için ek çalışmalara gerek vardır.*

Anahtar kelimeler: Non alkolik yağlı karaciğer hastalığı, genetik, hepatosteatoz, HLA

Address for correspondence: Mehmet ÇELİKBİLEK
Erciyes Üniversitesi Tıp Fakültesi Hastanesi Gastroenteroloji Bilim Dalı
Talas / Kayseri, Turkey
Fax: + 90 352 437 52 73
E-mail: drcelikbilek@yahoo.com

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is increasingly recognized as the most common cause of chronic liver disease worldwide (1). The disease is a common clinicopathological condition characterized by significant lipid deposition in the hepatocytes of the liver parenchyma in the absence of alcohol abuse, contributing medications and viral hepatitis. It comprises a disease spectrum that includes variable degrees of simple steatosis (fatty liver), non-alcoholic steatohepatitis (NASH) and cirrhosis (2). NASH is characterized by hepatocyte injury, inflammation and fibrosis, which can lead to cirrhosis. Recent evidence suggests that simple steatosis may also have the potential to progress to fibrosis and cirrhosis-like NASH (3,4).

Although the exact pathogenesis of NAFLD remains poorly understood, the two-hits theory is widely accepted to explain the progressive liver injury (5). The first hit is lipid accumulation in hepatocytes by insulin resistance and the second is oxidative stress, which causes the peroxidation of hepatocyte membrane lipid. NAFLD is strongly associated with obesity, insulin resistance, hypertension, hyperlipidemia, coronary artery disease, obstructive sleep apnea syndrome (OSAS), oxidative stress, and endothelial dysfunction, and is now regarded as the liver manifestation of the metabolic syndrome (6-9).

The genetic etiology of NAFLD is suggested by two family studies (10,11). This can be related with the well-known heritability of the risk factors for NAFLD. However, inter-ethnic variations in the prevalence of NAFLD and NAFLD-related cryptogenic cirrhosis strongly suggest that the genetic component of susceptibility to NAFLD may be important rather than hereditary risk factors (12-14). The human leukocyte antigen (HLA) is an objective genetic marker that may be implicated in the pathogenesis of a disease per se, or through a gene within the major histocompatibility complex that may contribute to disease susceptibility (15). The aim of our study was to evaluate the relationship between HLA and NAFLD.

MATERIALS AND METHODS

Study Population

In this cross-sectional study conducted at Baskent University Hospital between January 2000 and January 2008, data of 655 donor candidates were examined and data of 116 healthy candidates were also included (Figure 1). HLA class I and II antigen testing, blood tests (hepatitis B virus [HBV], hepatitis C virus [HCV], cytomegalovirus, human immunodeficiency virus, hepatic functions) and ultrasonography were routinely performed on all donor candidates.

Exclusion criteria were body mass index >30, alcohol intake, medication intake, positive results for viral seromarkers, and presence of any known systemic disease like diabetes mellitus, coronary artery disease, hyperlipidemia, or hypertension.

Diagnosis of NAFLD

NAFLD was diagnosed in 66 individuals with ultrasonography, and 50 individuals whose ultrasonography did not show hepatosteatosis comprised the control group. The diagnosis of NAFLD was based on increased liver echotexture on ultrasonography (Siemens Antares [Erlangen, Germany]) compared with the kidneys, vascular blurring and deep attenuation (16). Fat infiltration in the liver was described in three ultrasonographic stages using published criteria (17,18). The liver was considered to be normal if there was normal hepatic echotexture and normal beam attenuation. Mild steatosis was identified by a minimal increase in echogenicity of the liver parenchyma with a slight decrease in definition of the portal vein walls and minimal or no posterior beam attenuation. Severe steatosis was identified by grossly increased hepatic parenchymal echotexture that permitted visualization of the main portal vein walls alone. Smaller venules were not visualized, and there

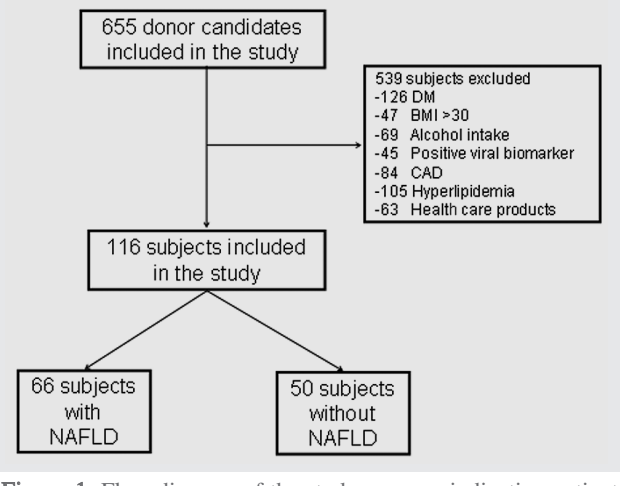

Figure 1. Flow diagram of the study process, indicating patient selection. DM, Diabetes mellitus; BMI, body mass index; CAD, coronary artery disease; NAFLD, nonalcoholic fatthy liver disease.

was increased posterior beam attenuation. Moderate steatosis was identified by hepatic echogenicity, portal venous definition and beam attenuation between mild and severe parameters. Patients with moderate and severe steatosis were included in this study.

HLA Analysis

HLA class I antigens (A1, A2, A3, A9, A11, A23, A24, A25, A26, A29, A30, A31, A32, A33, A34, A68, B7, B8, B13, B18, B27, B35, B38, B39, B40, B41, B44, B45, B47, B49, B50, B51, B52, B53, B55, B56, B57, B58, B60, B61, B62, B63, B64, B65, B75, Bw4, Bw6, Cw1, Cw3, Cw4, Cw6, Cw7, Cw9) were analyzed using the microlymphocytotoxicity test with the standard NIH method using a panel of commercial antisera (19). HLA class II antigens (DRB1*01, DRB1*0301, DRB1*0302, DRB1*04, DRB1*07, DRB1*0701, DRB1*08, DRB1*10, DRB1*1001, DRB1*11, DRB1*1101, DRB1*12, DRB1*13, DRB1*14, DRB1*15, DRB1*1501, DRB1*16, DRB3, DRB3*01, DRB3*03, DRB4*01, DRB5, DRB5*01, DRB11, DQB1*02, DQB1*04, DQB1*05, DQB1*06, DQB1*0601, DQB1*08, DQB1*0301, DQB1*0302, DQB1*0303, DQB1*00301, DR1, DR4, DR7, DR8, DR10, DR11, DR13, DR14, DR15, DR16, DR17, DR51, DR52, DR53, DQ2, DQ4, DQ5, DQ6, DQ7, DQ8, DQ9) were determined by the polymerase chain reaction sequence specific oligonucleotide method (PCR-SSO), a low-resolution molecular method, using a commercial kit (20).

Statistical Analysis

Statistical analysis was performed with SPSS version 13.0 for Windows. The differences between continuous variables were expressed as mean ± SD. Comparisons between continuous variables were performed with a non-parametric Mann–Whitney U test, whereas a chi-square test was performed for the comparison of the proportions of each categorical variable between the patients and controls. A probability value <0.05 was considered significant.

RESULTS

The demographic and clinical characteristics of the study population are presented in Table 1. The data of 116 donor candidates were analyzed; 66 candidates were diagnosed as NAFLD. The median age of the NAFLD group was 53.8 years (range, 32-77). In the NAFLD group, 33 (50%) candidates were female and 33 (50%) were male. Fifty candidates made up the control group and their median age was 44.6 years (range, 26-71). In the control group, 30 (60%) candidates were female and 20 (40%) were male.

Table 1. Demographic and clinical characteristics of the study population

	NAFLD group (n=66)	Control group (n=50)	P value
Male/Female, n	33/33	20/30	0.286
Median age, years, range	53.8 (32-77)	44.6 (26-71)	0.000
Median AST IU/L, range	22.8 (13-39)	18.6 (8-30)	0.003
Median ALT IU/L, range	27.1 (9-68)	20 (7-41)	0.009
HLA-*B65*, %	28.8	0	0.000
HLA-*DQ5*, %	40.7	16.1	0.038

When we compared the antigen frequencies of the NAFLD group with the control group, HLA-B65 was expressed significantly more in the NAFLD group. While we found HLA-B65 in 19 (28.8%) candidates in the NAFLD group, it was not found in any of the control candidates (p<0.001). Moreover, the prevalence of DQ5 antigens was significantly high (40.7%) in the NAFLD group compared to the control group (16.1%) (p<0.05).

As expected, the alanine aminotransferase (ALT) value was significantly higher in the NAFLD group (27.1 IU/L vs 20 IU/L, p<0.05). The aspartate aminotransferase (AST) value did not differ in the control and study groups (22.8 IU/L vs 18.6 IU/L, p=0.286).

DISCUSSION

The salient findings of the present study are: i- HLA might have a role in the etiopathogenesis of NAFLD, ii- HLA-B65 might be an independent risk factor for the development of NAFLD, and iii- HLA-DQ5 might also be an independent risk factor for the development of NAFLD.

The hypothesis that HLA could be involved in the development of this disorder derives from family studies and inter-ethnic variations in the prevalence of NAFLD and NAFLD-related cryptogenic cirrhosis (10-14). HLA associations have been shown in different ethnic populations with respect to HBV and HCV infection susceptibility, protection, disease severity, interferon treatment response, and response to vaccination. HLA-DRB1*11/*12 alleles and DQB1*0301 are related with HBV persistence worldwide. HLA allele asso-

ciation with interferon treatment response seems to differ for chronic HBV and HCV infections in global populations (21). HLA-DR13 is associated with HBV clearance (22). HLA-DRB1*1101 and DQB1*0301 alleles are related with reduced risk of developing chronic HCV infection (23). The relation of HLA alleles with autoimmune hepatitis, sclerosing cholangitis and primary biliary cirrhosis have been researched in the literature, and associations differ with ethnicity and geographical location (24). Yoshizawa et al. (25) demonstrated an association between HLA and OSAS, which is related with NAFLD. Their study showed an increased prevalence of HLA-A2 in patients with OSAS as compared with healthy individuals.

The relation between HLA and alcoholic liver disease has also been researched in many studies. The associations of HLA-B40 and DRW9 with Japanese alcoholic liver cirrhosis have been shown (26). Doffoel et al. (27) also demonstrated that HLA-B15 and HLA-DR4 were significantly higher in an alcoholic cirrhosis group than in controls. Mills et al. (28) illustrated no association between HLA antigens and alcoholic liver disease. The results of our study indicated that NAFLD may represent another liver disease associated with HLA.

The diagnosis of NAFLD requires evidence of fatty infiltration of the liver in the absence of excessive alcohol consumption. In this study, we also excluded patients with alcohol intake. To reduce the influence of possible confounding factors, we excluded diseases known to be associated with NAFLD like obesity, diabetes mellitus, coronary artery disease, hyperlipidemia, and metabolic syndrome.

The association of HLA-B65 and DQ5 with NAFLD could indicate one of several underlying possibilities. The first is that HLA-B65 may be responsible for disease susceptibility. Another possible explanation is a linkage disequilibrium between the disease locus and the HLA antigen. This means that there is a disease locus that is within the HLA region of the chromosome but is not itself part of the HLA region.

This study has some limitations. The first limitation was the small sample size. The second was that the diagnostic method depended on ultrasonography and the exclusion of other, secondary causes of chronic liver disease, but was not confirmed by liver biopsy. Although liver biopsy is currently the gold standard for distinguishing NAFLD forms, for assessing the severity of damage and prognosis, NAFLD can be detected as a bright liver on ultrasonography and it is possible to perform routinely. Moreover, liver ultrasonography has proven to be a sensitive, accurate and convenient diagnostic tool in detecting steatosis. Its sensitivity ranges from 60-94% and its specificity from 84-95% (16,29).

In conclusion, multiple factors may be involved in the pathogenesis of NAFLD, such as genetics, and HLA-B65 and DQ5 were significantly different in the NAFLD group than in the control group. Our study may be helpful in understanding the pathogenesis and epidemiology of this common disorder. This is the first attempt to study HLA in NAFLD patients in the literature and should be considered as a preliminary report. The association of HLA with fatty liver progression to NASH and cirrhosis may be considered in future studies.

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