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Liver injury correlates with biomarkers of autoimmunity and disease activity and represents an organ system involvement in patients with systemic lupus erythematosus

Yuxin Liu [a], Jianghong Yu [a], Zachary Oaks [a], Ivan Marchena-Mendez [a], Lisa Francis [a], Eduardo Bonilla [a], Phillip Aleksiejuk [a], Jessica Patel [a], Katalin Banki [b], Steve K. Landas [b], Andras Perl [a,*]

[a] Division of Rheumatology, Department of Medicine, State University of New York, Upstate Medical University, Syracuse, NY, USA
[b] Division of Rheumatology, Department of Pathology, State University of New York, Upstate Medical University, Syracuse, NY, USA

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ABSTRACT

Liver disease (LD), defined as ≥2-fold elevation of aspartate aminotransferase (AST) or alanine aminotransferase (ALT), was examined in a longitudinal study of systemic lupus erythematosus (SLE) patients. Among 435 patients, 90 (20.7%) had LD with a greater prevalence in males (15/39; 38.5%) than females (75/396; 18.9%; $p = 0.01$). SLE disease activity index (SLEDAI) was greater in LD patients (7.8 ± 0.7) relative to those without (5.8 ± 0.3; $p = 0.0025$). Anti-smooth muscle antibodies, anti-DNA antibodies, hypocomplementemia, proteinuria, leucopenia, thrombocytopenia, and anti-phospholipid syndrome were increased in LD. An absence of LD was noted in patients receiving rapamycin relative to azathioprine, cyclosporine A, or cyclophosphamide. An absence of LD was also noted in patients treated with N-acetylcysteine. LFTs were normalized and SLEDAI was diminished with increased prednisone use in 76/90 LD patients over 12.1 ± 2.6 months. Thus, LD is attributed to autoimmunity and disease activity, it responds to prednisone, and it is potentially preventable by rapamycin or N-acetylcysteine treatment.

1. Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease that causes inflammation in multiple organ systems with diverse clinical manifestations [1]. It has been reported that patients with SLE have a 9.3% to 59.7% chance of developing abnormal liver function tests (LFT) during follow-up periods of multiple years [2–5]. Two commonly measured LFTs are aspartate transaminase (AST) and alanine transaminase (ALT). These transaminases participate in amino acid metabolism and are normally found at low levels in plasma serum. However, upon hepatocyte damage, these liver enzymes are released, and abnormal levels can be detected in the circulation [6]. In addition to indicating liver damage, the ratio of AST to ALT can help differentiate the etiology [7].

Management of SLE patients with persistent AST and ALT elevations is challenging. Physicians may be confronted with long-lasting abnormal liver enzymes, which cannot be explained by any obvious causes after excluding viral hepatitis, alcohol toxicity, and potentially harmful drugs. Discerning the cause of liver dysfunction and the safety of immunosuppressant treatments are difficult in these patients. Although the association between SLE and liver disease has been observed on multiple occasions, the relationship of liver disease to co-morbidities and drugs has not been well established. Our study has been initiated to determine the causes of liver disease with a focus on the contributions of SLE disease activity and medication use. This initiative was prompted by the common dilemma that the clinician face in daily practice with respect to handling of liver enzyme elevations. Recent studies set the threshold for drug-induced liver injury at a 2-fold elevation of ALT or AST, depending on the patient population involved [8,9]. In immunocompromised patients, such as those infected by human immunodeficiency virus (HIV) or hepatitis C virus (HCV), the threshold of liver injury was set at a 2-fold elevation of ALT or AST [8,9]. Therefore, we

Abbreviations: AIH, autoimmune hepatitis; AMA, anti-mitochondrial antibody; Anti-DNA, anti-double stranded DNA antibody; ANA, antinuclear antibody; Anti-TNF, tumor necrosis factor blocker; ALT, alanine aminotransferase; APLA, anti-phospholipid antibody; APS, anti-phospholipid syndrome; AST, aspartate aminotransferase; AZA, azathioprine; BMI, body mass index; C3, complement factor; C4, complement factor 4; CPK, creatine phosphokinase; CsA, cyclosporine A; CTX, cyclophosphamide; HCQ, hydroxychloroquine; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HIV, human immunodeficiency virus; iLD, intermediate liver disease; LD, liver disease (LD); LFT, liver function test; MMF, mycophenolate mofetil; MPA, mycophenolic acid; mTOR, mechanistic target of rapamycin; NAC, N-acetylcysteine; NSAID, non-steroidal anti-inflammatory drug; PBL, peripheral blood lymphocytes; PRED, prednisone; Rapa, rapamycin/sirolimus; SLE, systemic lupus erythematosus; SLEDAI, systemic lupus erythematosus disease activity index; SMA, anti-smooth muscle antibody; TSH, thyroid-stimulating hormone; WBC, white blood cell.

* Corresponding author at: SUNY Upstate Medical University, 750 East Adams Street, Syracuse, NY 13210, USA.
E-mail address: perla@upstate.edu (A. Perl).

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have undertaken a longitudinal study of ALT and AST elevations in SLE patients by excluding subjects with alcohol abuse, hepatitis and human immunodeficiency virus infection, or thyroid disease, all of which can cause liver disease independent of SLE [10–12]. None of these confounding factors have been previously excluded in previous studies of LFT elevation with respect to disease activity and medication use in patients with SLE. The results of this conservatively defined longitudinal study of 435 SLE patients indicate that LD, which is delineated as a ≥2-fold elevation of ALT or AST, may represent a manifestation of lupus disease activity and respond to continued immunosuppression and introduction of prednisone rather than caused by drug toxicity.

2. Methods

2.1. Human subjects

Patients who satisfied the American College of Rheumatology criteria for a definitive diagnosis of SLE [13,14] among those seen and treated at SUNY Upstate Medical University Hospital from October of 1999 to December of 2011 were included in this study. The clinical protocol was approved by the Institutional Review Board. All patients of our lupus cohort are screened for antibodies to hepatitis A, B, or C virus. Patients with evidence of hepatitis A, B, or C virus infection, human immunodeficiency virus (HIV) infection, IgM-positive recent parvovirus B19 infection, and those with a history of alcohol abuse have been excluded. Alcohol abuse diagnosis was made using the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (*DSM-5*) and definition of a problematic pattern of alcohol consumption [15]. Allowable alcohol consumption was considered as an average of one drink per day. One drink was defined as 12 oz of beer, 4 oz of wine, or 1 oz of liquor which do not elicit AST or ALT elevation [16]. To rule out non-hepatic disease as a cause of abnormal liver function tests, SLE patients who possessed elevated creatine phosphokinase (CPK) and thyroid-stimulating hormone (TSH) laboratory values were also excluded from analysis. We defined liver disease (LD) as a 2-fold or greater elevation of serum AST or ALT above the upper limit of the normal range. Patients with a greater than normal, but less than 2-fold elevation in AST or ALT were defined to have intermediate liver disease (iLD). The remaining patients who never had an elevated LFT value were classified as normal. As markers of autoimmune hepatitis (AIH) and primary biliary cirrhosis, anti-smooth muscle antibody (SMA) and anti-mitochondrial antibody (AMA) were assessed. Patient demographics, such as gender, age at the time of LD, ethnicity, and body mass index (BMI) were noted. We analyzed the prevalence of liver disease with respect to diabetes. 27 patients had diabetes, 5 of them type 1. 18 patients were treated with insulin, while 9 patients were only treated with oral anti-diabetic medications, 6 with metformin, 2 with glipizide, 2 with glyburide, and 2 with sitagliptin. The medications taken by SLE patients with LD were recorded for the day when the patient exhibited a 2-fold elevation of liver enzymes and for the next follow-up date when the patient's LFT have normalized. In addition, we reviewed liver imaging studies and liver biopsies when available.

2.2. Routine laboratory tests

AST (normal range: males, <37 U/L; females, <31 U/L), ALT (normal range: males < 41 U/L; females < 31 U/L), CPK (normal range: males, 20–200 U/L; females, 20–180 U/L), TSH (normal range: 0.270–4.200 μIU/mL), C3 (normal range: 90–180 mg/dL), and C4 (normal range: 10–40 mg/dL) were measured on a Roche/Hitachi Modular Analyzer (*Roche Diagnostics*, Indianapolis, IN). Platelets (normal range: 150–400 K/μL) and WBC (normal range: 4.0–10.0 K/μL) were counted on a Beckman–Coulter LH 750 Hematology Analyzer (Brea, CA). Lupus anticoagulants were assessed by Staclot LA (delta < 10 s), Staclot® dRVV (<1.2 normalized ratio) manufactured by Stago Diagnostics (Parsippany, NJ, USA). Platelet neutralization assay has been developed in house (delta < 1 s) using a STA-R Evolution instrument by Diagnostica Stago Diagnostics. Cardiolipin and β2-glycoprotein 1 (β2-GP1) antibodies were measured by Quest Diagnostics (Madison, NJ). Antinuclear antibody (ANA; normal range >1:50 dilution) was detected in the HEp-2 Test System by Zeus Scientific (Raritan, NJ). The immunofluorescent ANA test was used for diagnosis of all patients [17]. Anti-mitochondrial antibody (AMA; normal range > 1:50 dilution) and anti-smooth muscle antibody (SMA; normal range > 1:50 dilution) were measured using NOVA Lite® ANA Plus Mouse Kidney & Stomach assay manufactured by Inova Diagnostics (San Diego, CA). Hepatitis viral antibody tests were performed on an ARCHITECT *i*1000SR Immunoassay Analyzer manufactured by Abbott Diagnostics (Abbott Park, IL).

2.3. Biomarkers of lupus disease activity

As markers of active SLE, the presence of proteinuria, glomerulonephritis, anti-double stranded DNA antibodies (anti-DNA), hypocomplementemia, leukopenia, thrombocytopenia, and antiphospholipid antibodies (APLA), lupus anticoagulant, and antiphospholipid syndrome (APS) was recorded. APS was confirmed by a clinical thrombotic event and positive tests for cardiolipin or β2-GP1 antibodies or lupus anticoagulant that was persistent for ≥12 weeks [18]. SLE disease activity was assessed by using systemic lupus erythematosus disease activity index (SLEDAI) [19]. Patients that were positive for proteinuria had urine protein levels greater than 0.5 g/day [19]. Hypocomplementemia was classified as low C3 or low C4. Patients with leukopenia or thrombocytopenia had white blood cell (WBC) counts less than 4000/L and platelet counts less than 100,000/L, respectively.

2.4. Statistical analyses

Statistical assays were performed with two-tailed χ^2-test, Fisher's exact test, and correlation analysis using the GraphPad Prism Version 5 software (San Diego, CA). A two-tailed p value <0.05 was considered significant.

3. Results

3.1. Prevalence of LD is increased in male SLE patients

20.7% (90/435) of SLE patients met our criteria of LD, 29.0% (126/435) had iLD, and 50.3% (219/435) had normal AST and ALT (Table 1). Assessment of demographic differences included ethnicity, age, and gender. Among these parameters, ethnicity did not influence the prevalence of LD or iLD in SLE (Table 1). Out of 435 SLE patients, 39 were male and 396 were female. Thus, 38.5% of male patients had LD, while only 18.9% of female patients had LD relative to subjects with normal LFTs (χ^2 $p = 0.01$; Table 1). The prevalence of LD was also increased in males relative to subjects with iLD (χ^2 $p = 0.02$; Table 1). Age was not significantly different between the three cohorts of LD, iLD, and normal LFTs. However, when comparing patients based on gender and age it revealed that subjects with iLD (45.2 ± 1.1 years) were slightly older than those with LD among females (41.1 ± 1.8 years; $p = 0.045$; Table 2). The average AST/ALT ratio was 1.21 ± 0.04 in all patients, and it was not affected by age, ethnicity, or gender (Table 2). Body mass index (BMI) was not significantly different between patients with LD, iLD, and normal LFTs (Table 2). No significant differences in BMI were noted either when male and female patients were separately analyzed (Table 2). As shown in Table S1, concurrent diabetes did not influence the prevalence of liver disease.

3.2. LD is associated with lupus disease activity characterized by increased SLEDAI, anti-DNA and hypocomplementemia

The greatest differences in biomarkers of lupus disease activity were observed when the LD cohort was compared to lupus patients with normal LFTs. SLEDAI was greater in LD patients (7.8 ± 0.7) relative to those