The role of liver biopsy in the differential diagnosis of autoimmune hepatitis and drug-induced liver injury

Otoimmun hepatit ve ilaca bağlı karaciğer hasarı ayırıcı tanısında karaciğer biyopsisinin rolü

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Background and Aims: *Our goal was to determine the histological properties, potential differentiating histological markers and the value of a liver biopsy in the differential diagnosis of autoimmune hepatitis and drug-induced liver injury.* ***Materials and Methods:*** *Forty-seven liver biopsies from patients with clinically well-defined autoimmune hepatitis and drug-induced liver injury were assessed. Only very highly probable or highly probable cases, according to the Roussel Uclaf Causality Assessment Method, were included in the study. The laboratory results were reviewed retrospectively.* ***Results:*** *The blood levels of immunoglobulin G, iron and ferritin were significantly different between patients with autoimmune hepatitis and drug-induced liver injury. Statistically, there was no significant difference between the groups regarding lymphocyte and eosinophil levels, piecemeal necrosis, confluent necrosis and zone 3 necrosis, badge formation levels or the stage of fibrosis. However, there was a significant difference between the two groups in plasma cell levels.* ***Conclusions:*** *Only plasma cell levels are pathologically useful in distinguishing autoimmune hepatitis and drug-induced liver injury. Therefore, serum ferritin, iron and immunoglobulin G values, in addition to plasma cells levels, can be used in differential diagnosis of autoimmune hepatitis and drug-induced liver injury. The percentage of monocytes is significantly different between these groups, and this finding should be further investigated.*

Key words: *Autoimmune hepatitis; differential diagnosis; drug-induced liver injury*

Giriş ve Amaç: *Amacımız otoimmün hepatit ve ilaca bağlı karaciğer hasarının ayırıcı tanısında histolojik özellikleri, potansiyel ayırt ettirici histolojik belirteçleri ve karaciğer biyopsisinin değerini incelemektir.* ***Gereç ve Yöntem:*** *Klinik olarak iyi tanımlı otoimmün hepatit ve ilaca bağlı karaciğer hasarı olan vakalardan alınan 39 karaciğer biyopsisini değerlendirdik. Roussel Uclaf Causality Assessment Method kullanılarak, sadece çok yüksek olasılıklı, ya da yüksek olası olarak değerlendirilen vakalar çalışmaya dahil edildi. Laboratuvar sonuçları retrospektif olarak elde edildi.* ***Bulgular:*** *Otoimmüun hepatit ve ilaca bağlı karaciğer hasarı olan hastalar arasında immünglobulin G, demir ve ferritin düzeyleri arasında istatistiksel olarak anlamlı fark bulunmuştur. Lenfosit, eozinofil, interface hepatit, lobüler hepatit, zon 3 nekroz, birleşme nekrozu, rozet formasyonu ve fibrozis derecesi açısından gruplar arasında fark yoktur. Plazma hücresi seviyesi bakımından iki grup arasında istatistiksel olarak anlamlı bir farklılık vardır.* ***Sonuç:*** *Patolojik olarak sadece plazma hücre düzeyleri otoimmün hepatit ve ilaca bağlı karaciğer hasarını ayırt etmede yardımcıdır. Bu nedenle, otoimmün hepatit ve ilaca bağlı karaciğer hasarı ayırıcı tanısında plazma hücre düzeylerine ek olarak serum ferritin, demir ve immünglobulin G düzeyleri kullanılabilir. İki grup arasında monosit oranları anlamlı dercede farklıdır ve bu bulgunun daha ileri araştırmalarla desteklenmesi gereklidir.*

Anahtar kelimeler: *Otoimmün hepatit, ilaca bağlı karaciğer hasarı, ayırıcı tanı*

INTRODUCTION

Establishing a clinical diagnosis of autoimmune hepatitis (AIH) and drug- induced liver injury (DILI) are difficult because these two diseases demonstrate both clinically and histopathologically heterogeneous findings (1). Given that both diseases are identified using immunological assays, they are similar in clinical and histopathological specifications (2). Even though there is only one factor that induces the disease, the clinical manifestations of DILI vary. For example, some drugs (statins, minocycline, nitrofurantoin and infliximab) can cause typical hepatocellular and cholestatic liver injury, and, in other cases, autoimmune reactions are induced or human leucocyte antigen haplotypes are seen accompanying autoimmune hepatitis. In addition, it is generally difficult to clinically distinguish between AIH and DILI (1,2). In some circumstances, the clinical distinction between the two diseases may be impossible to determine due to the confounding role of potential drug interactions (3). Early diagnosis and appropriate treatment are critical in both disease processes. The prompt use of immunosuppressants can

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decrease disease activity in patients with idiopathic AIH. Similarly, the early identification and discontinuation of a drug can prevent progressive liver injury in patients with DILI (1,4). The use of liver biopsy in differentiating AIH from DILI has produced contradictory results. Recently, a histological classification of DILI was reported with a differential diagnosis and histological specifications corresponding to both injury patterns (3). The characteristic histological attributions of AIH are well documented in the literature (5). Interstitial hepatitis, lymphocytic (lymphoplasmacytic in portal tracts) efflux lying towards the lobule, emperipolesis (e.g., intact lymphocytes in the hepatocyte cytoplasm) and badge formation of hepatocytes are the most commonly known signs of AIH and are part of recently published simplified AIH diagnostic criteria (6). However, there is no definite histological finding to distinguish between DILI and AIH (7). Therefore, the role of liver biopsy in differentiating AIH from DILI is not clear (8); it may be useful in separating AIH and DILI on a histological spectrum.

In this study, a blinded histological assessment was performed on the liver biopsies of patients with well-defined AIH and DILI, in accordance with standard procedures (9). Our goal was to determine the histological properties, potential differentiating histological and laboratory markers, and the value of liver biopsy in establishing a diagnosis between AIH and DILI. In addition, a subgroup analysis was performed to compare the histological properties between AIH and DILI and discover potential markers that could be used to facilitate diagnosis.

MATERIALS and METHODS

Forty-seven liver biopsies from patients with clinically well-defined AIH and DILI were assessed. AIH was diagnosed by detecting the presence of autoantibodies and/or gamma globulins compatible with the disease, and after excluding other aetiologies. DILI was diagnosed using the Roussel Uclaf Causality Assessment Method (RUCAM), an assessment that was standardized for every region, and/or clinical judgments. Only very highly probable or highly probable cases, according to the RUCAM, were included in the study. The following laboratory results were reviewed retrospectively: viral hepatitis marker anti-hepatitis A virus (anti-HAV) immunoglobulin M (IgM), hepatitis B surface antigen (HBsAg), hepatitis B virus (HBV) DNA polymerase chain reaction (PCR), anti-hepatitis C virus (anti-HCV) HCV RNA, anti-hepatitis E virus (anti-HEV), anti-cytomegalovirus (anti-CMV) IgM, anti-CMV immunoglobulin G (IgG), monospot test, Paul-Bunnel test, autoantibodies [antinuclear antibodies (ANA), anti-mitochondrial antibodies (AMA), anti-smooth muscle antibodies (ASMA) and liver kidney microsomal 1 (LKM1) antibodies] as well as alpha-1 antitrypsin, ferritin, ceruloplasmin and serum/urine copper ratio, which helped exclude metabolic liver disease.

Statistical analyses were performed using SPSS version 17. The variables were investigated using visual (histograms, probability table) and analytic methods (Kolmogorov-Smirnov/ Shapiro-Wilk test) to determine whether their range was normal. Non-parametric variables were assessed as medians and ranges (min-max), and the differences in median values between the two groups were determined using the Mann-Whitney U test. Other parametric variables were determined as the mean±standard deviation, and Student's t-test was used to compare independent data. Dichotomous variables were compared using the Fischer accuracy test or the Pearson's chi-square test. All tests were bidirectional, and the level of significance was set at 5%.

RESULTS

The median aspartate aminotransferase (AST) value was 712 U/L (range, 97-1234) for patients with AIH and 714 U/L (range, 43- 4387) for patients with DILI. The median alanine aminotransferase (ALT) value was 740 U/L (range, 112-1435) for AIH patients and 743 U/L (range, 41-4419) for patients with DILI. The median alkaline phosphatase (ALP) value was 159 U/L (range, 45-356) for patients with AIH and 145 U/L (range, 56-517) for patients with DILI. The mean immunoglobulin G (IgG) value was 2024±504 g/dL for AIH patients and 988±555 g/dL for patients with DILI.

The median gamma glutamine transferase (GGT) value was 105 U/L (range, 36-231) for patients with AIH and 132 U/L (range, 42-497) for patients with DILI. The values of AST, ALT, IgG, iron, ferritin and monocyte (MO) were statistically significantly different between patients with AIH and those with DILI (Table 1).

The detailed antinuclear antibody (ANA) frequencies of patients with AIH and DILI are shown in Table 2. Fifty percent of AIH patients and 22.2 % of DILI patients exhibited ANA positivity at a titre of 1/40; 77.8 % of the DILI patients exhibited ANA negativity Table 2.

Histopathologic and morphologic comparisons between patients with AIH and DILI are shown on Table 3. The median value of lymphocyte levels among patients with autoimmune hepatitis were similar to that of toxic hepatitis patients. Statistically, there was no significant difference between the groups regarding lymphocyte levels.

The median plasma cell levels among patients with autoimmune hepatitis and among patients with toxic hepatitis were 1 (1-2) and 1 (0-2), respectively, and there was no difference between groups. The median eosinophil levels were 0-5 (0-2) in patients with AIH, and 1 (0-2) in toxic hepatitis patients; there was no difference between the groups. The median values of piecemeal necrosis levels among patients with AIH and DILI were 2 (0-3) and 1 (0-2), respectively. The median value of lobular hepatitis level was 2 (1-3) in both groups. The median values of zone 3 necrosis levels between patients with AIH and DILI were 1 (0-1) and 0 (0-2), respectively. The median levels of confluent necrosis between the AIH patients and DILI patients were 0 (0-1) and 0 (0-2), respectively. The median values of badge formation levels between patients with AIH and DILI were 1.5 (0-3) and 1 (0-2), respectively. The median stage of fibrosis among patients with autoimmune hepatitis and toxic hepatitis were 2.5 (0-6) and 1 (0-6), respectively.

There was no difference between groups regarding piecemeal necrosis, lobular hepatitis level, zone 3 necrosis, confluent necrosis, badge formation and median stage of fibrosis.

DISCUSSION

In daily practice, it is difficult to distinguish clinically between patients with drug-induced hepatitis and those with autoimmune hepatitis. It is especially difficult to distinguish between AIH and DILI when AIH is with negative

Table 1. Comparison of demographics, seropositivity, AIH score and histology among autoimmune hepatitis (AIH) and drug-induced liver injury (DILI) patients

	AIH	DILI	p-value
Patients, n (%)	20 (42.6)	27 (57.4)	
Age, years	49±16	46±10	>0.05
Gender			
Male	3 (15.0)	5 (18.5)	>0.05
Female	17 (85.0)	22 (81.5)	
ANA positive, n (%)	20 (100.0)	6 (22.2)	<0.0001
LKM-1 positive, n (%)	6 (30.0)	0 (0.0)	0.004
Simplified AIH score	7 (6-8)	-	-
Probable or accurate score	17.5 (14-22)	-	-
AMA positive, n (%)	2 (10.0)	-	-
AST (U/L), median (range)	712 (97-1234)	714 (43-4387)	0.010
ALT (U/L), median (range)	740 (112-1435)	743 (41-4419)	0.013
ALP (U/L), median (range)	159 (45-356)	145 (56-517)	>0.05
GGT (U/L), median (range)	105 (36-231)	132 (42-497)	>0.05
LDH (U/L), median (range)	475 (167-716)	513 (166-3513)	>0.05
IgG (g/dL), mean±SD	2024±504	988 ± 555	<0.0001
CRP (mg/L), median (range)	1.29 (0.40-4.40)	1.19 (0.01-3.70)	>0.05
Total Protein (g/dL), mean±SD	6.4±0.9	6.7±1.0	>0.05
Albumin (g/dL), mean±SD	3.2±0.5	3.4±0.7	>0.05
T. Bilirubin (mg/dL), median (range)	3.2 (0.5-14.2)	4.5 (0.5-41.5)	>0.05
D. Bilirubin (mg/dL), median (range)	2.1 (0.1-13.0)	3.2 (0.2-34.9)	>0.05
Iron (µg/dL), median (range)	62 (11-93)	76 (16-205)	0.019
Total Iron binding capacity (TIBC) (µg/dL), median (range)	278 (247-381)	292 (214-427)	>0.05
Ferritin (µg/L), median (range)	34 (10-472)	56 (12-1388)	0.031
Haemoglobin (g/dL), median (range)	13.9 (11.0-15.1)	13.9 (7.3-16.6)	>0.05
MCV(fL) median (range)	91 (88-94)	90 (68-97)	>0.05
ESR (mm/h), median (range)	12 (6-21)	12 (2-69)	>0.05
Monocyte (%), median (range)	11 (9-13)	12 (6-20)	0.010
INR, median (range)	1.1 (0.9-1.7)	1.1 (0.8-4.3)	>0.05

ANA; Antinuclear antibody, LKM; Liver-kidney microsomal, AMA; Anti-mitochondrial antibody, AST; Aspartate transaminase, ALT; Alanine transaminase, ALP; Alkaline phosphatase, GGT; Gamma-glutamyl transferase, LDH; Lactate dehydrogenase, CRP; C-reactive protein, ESR; Erythrocyte sedimentation rate, MCV; Mean corpuscular volume, INR; International normalised ratio.

Table 2. Frequency of ANA among AIH and DILI patients

	AIH (n=20)	DILI (n=27)
Anti-nuclear antibody (ANA)		
Negative, n (%)	-	21 (77.8)
1/40, n (%)	10 (50)	6 (22.2)
1/80, n (%)	5 (25)	-
1/160, n (%)	4 (20)	-
1/320, n (%)	1 (5)	-
Liver- kidney microsomal (LKM)		
Negative, n (%)	14 (70)	27 (100)
1/40, n (%)	5 (25)	-
1/160, n (%)	1 (5)	-

autoantibodies and DILI is with ANA positivity (10,11). In recent years, the changing definition of AIH discussed in many reports further elaborates on the situation (11,12). Due to a difference in treatment approaches for both conditions and, especially because of the need to discontinue drug therapy in DILI cases, these two diseases having similar histopathological patterns and laboratory survey results should be differentiated (13,14). In this study, in addition to currently used antibodies, we attempted to determine histopathologic differences between the two diseases. Thus, we compared lymphocyte, plasma cells, interface hepatitis, lobular hepatitis, zone 3 necrosis, confluent necrosis, badge formation and fibrosis levels. Unexpectedly, we determined that eosinophil infiltration, a characteristic property of drug-induced hepatitis, is useless in distinguishing between the two diseases. Eventually, as indicated in Table 3, we determined that the plasma cell count was the only parameter that differed between the groups. These findings are consistent with the series of articles published by Suzuki et al., in 2010 and Björnsson et al, in 2010 (2,15). However, this is contrary to the study published by Lewis et al., which suggests that there is some significance between the two groups concerning interface hepatitis (16). The difference in results may be attributed to the quality of the pathologic preparations selected.

The interesting finding in this study was the difference in the monocyte percentage levels between the two groups. According to the literature, no study assessed this finding.

This study has some limitations. The main advantage of this study was that the pathological investigation was performed by one pathologist, and the main disadvantage was the small sample size.

In conclusion, plasma cell levels, in addition to serum iron, ferritin and IgG levels, are helpful in distinguishing between autoimmune hepatitis and drug-induced liver injury. In addition to this finding, we are the first to report that the monocyte percentage levels were significantly different between these two groups and should be considered by researchers as an area of interest.

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We declare no conflict of interest.

Table 3. Histopathological and morphological comparison of AIH and DILI patients

	AIH (n=20)	DILI (n=27)	P value
Lymphocyte	2 (1 - 3)	1.5 (1 - 2)	>0.05
Plasma cell	**1 (1 - 2)**	**1 (0 - 2)**	**0,032**
Eosinophil	0.5 (0 - 2)	1 (0 - 2)	>0.05
Piecemeal necrosis (Interface activity)	2 (0 - 3)	1 (0 - 2)	>0.05
Lobular hepatitis	2 (1 - 3)	2 (1 - 3)	>0.05
Zone 3 necrosis	1 (0 - 1)	0 (0 - 2)	>0.05
Confluent necrosis	0 (0 - 1)	0 (0 - 2)	>0.05
Badge formation	1.5 (0 - 3)	1 (0 - 2)	>0.05
Fibrosis	2.5 (0 - 6)	1 (0 - 6)	>0.05

REFERENCES

1. Ju HY, Jang JY, Jeong SW, et al. The clinical features of drug-induced liver injury observed through liver biopsy: focus on relevancy to autoimmune hepatitis. Clin Mol Hepatol 2012;18:213-8.
2. Suzuki A, Brunt EM, Kleiner DE, et al. The use of liver biopsy evaluation in discrimination of idiopathic autoimmune hepatitis versus drug-induced liver injury. Hepatology 2011;54:931-9.

3. Czaja AJ. Acute and acute severe (fulminant) autoimmune hepatitis. Dig Dis Sci 2013;58:897-914.
4. Efe C. Drug induced autoimmune hepatitis and TNF-α blocking agents: Is there a real relationship? Autoimmun Rev 2013;12:337-9.
5. Efe C, Özaslan E, Purnak T, et al. Liver biopsy is a superior diagnostic method in some patients showing the typical laboratory features of autoimmune hepatitis. Clin Res Hepatol Gastroenterol 2012;36:185-8.
6. Efe C, Purnak T, Ozaslan E, Wahlin S. Drug-induced autoimmune hepatitis caused by anti-tumor necrosis factor α agents. Hepatology 2010;52:2246-7.
7. Schaefer EA, Pratt DS. Autoimmune hepatitis: current challenges in diagnosis and management in a chronic progressive liver disease. Curr Opin Rheumatol 2012;24:84-9.
8. Czaja AJ. Drug-induced autoimmune-like hepatitis. Dig Dis Sci 2011;56:958-76.
9. Ramachandran R, Kakar S. Histological patterns in drug-induced liver disease. J Clin Pathol 2009;62:481-92.
10. Efe C, Özaslan E, Kav T, et al. Liver fibrosis may reduce the efficacy of budesonide in the treatment of autoimmune hepatitis and overlap syndrome. Autoimmun Rev 2012;11:330-4.
11. Czaja AJ, Bayraktar Y. Non-classical phenotypes of autoimmune hepatitis and advances in diagnosis and treatment. World J Gastroenterol 2009;15:2314-28.
12. Efe C, Ozaslan E, Nasiroglu N, et al. The development of autoimmune hepatitis and primary biliary cirrhosis overlap syndrome during the course of connective tissue diseases: report of three cases and review of the literature. Dig Dis Sci 2010;55:2417-21.
13. Bach JF. Infections and autoimmune diseases. J Autoimmun 2005;5 (Suppl):74-80.
14. Czaja AJ. Emerging treatments for autoimmune hepatitis. Curr Drug Targets Inflamm Allergy 2002;1:317-26.
15. Björnsson E, Talwalkar J, Treeprasertsuk S, et al. Drug-induced autoimmune hepatitis: clinical characteristics and prognosis. Hepatology 2010;51:2040-8.
16. Lewis JH. Diagnosis: Liver biopsy differentiates DILI from autoimmune hepatitis. Nat Rev Gastroenterol Hepatol 2011;8:540-2.