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Associations of gender and a proxy of female menopausal status with histological features of drug-induced liver injury

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Abstract

Background & Aim: Gender and menopause may contribute to type and severity of drug-induced liver injury (DILI) by influencing host responses to injury. The aim of this study was to assess the associations of gender and female age 50 [a proxy of menopause] with histological features of liver injury in 212 adults enrolled in the Drug-Induced Liver Injury Network (DILIN) registry.

Methods: All participants had a causality score of at least 'probable', a liver biopsy within 30 days of DILI onset, and no prior chronic liver disease. Biochemical and histological injury types were classified as hepatocellular or cholestatic/mixed injury. The cohort was divided into three gender/age categories: men (41.0%), women <50 years (27.4%) and women ≥50 years of age (31.6%). Interaction of gender and age category (≥50 or not) was assessed.

Results: Hepatocellular injury was more prevalent in women <50 years vs. others (P=.002). After adjusting for biochemical injury types, black race and possible ageing effects, more severe interface hepatitis was noted in biopsies of women <50 years compared to those of men and women ≥50 years (P=.009 and P=.055 respectively). Compared to those of men, biopsies of women showed greater plasma cell infiltration, hepatocyte apoptosis, hepatocyte rosettes and lobular disarray but less iron-positive hepatocytes and histological cholestasis (P<.05). These associations persisted after excluding cases of amoxicillin/clavulanic acid, anabolic steroids or nitrofurantoin DILI which showed gender-specific distributions.

Conclusion: Gender and a proxy of menopause were associated with various features of inflammation and injury in DILI.

KEYWORDS

drug-induced liver injury, gender difference, hepatotoxicity, liver histology, menopause

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; ANA, antinuclear antibody; CI, confidence interval; CS/MIX, cholestatic/mixed; DILI, drug-induced liver injury; DILIN, drug-induced liver injury network; HC, hepatocellular; OR, odds ratio; ULN, upper limit normal.

1 | INTRODUCTION

Significant heterogeneity exists in clinical phenotypes of drug-induced liver injury (DILI). Initial biochemical presentations, autoimmune features, liver histology and clinical outcomes vary substantially among individuals who develop DILI, even when caused by the same agent.[1–4] Such heterogeneity suggests that how individuals respond to drug toxicity, cellular stress and tissue injury may contribute to the variable clinical phenotypes of DILI.

Gender and sex hormones influence drug metabolism and transport.[5,6] They also modulate host responses to injury; sexual dimorphism in cellular stress response, cell death, immune response, inflammation and tissue repair has been demonstrated in various systems. In particular, sexual dimorphism in the immune response has been well-documented in humans as well as experimental models.[7] Several recent experimental studies demonstrated sex differences in host responses to injury. An immune-mediated DILI model showed sex differences in immune response and inflammation: more severe hepatitis, more antibody production, and a higher level of pro-inflammatory hepatic cytokines in females vs. male mice.[8] In the halothane-induced liver injury mouse model, oestrogens reduce injury while progesterone exacerbates injury.[9,10] In an immune-mediated nephritis model more apoptosis occurred in females vs. more necrosis in males, and the administration of oestrogen to male mice induced apoptosis and inhibited necrosis.[11] Taken together, these findings suggest that gender and sex hormones may modulate host responses to liver injury insults and contribute to diverse clinical manifestation and severity of human DILI.

We hypothesized that gender and sex hormones modulate cellular stress responses, cell death, immune responses and inflammation in drug toxicity and may influence clinical and histological DILI manifestations in humans. We aimed to investigate the associations of gender and women's age (with ≥50 years as a surrogate for menopause) with histological features in acute phase of DILI, for the purpose of further refining the above-mentioned hypothesis. Our thorough descriptive analyses using different modelling approaches identified several histological features associated with gender (and women's age <50) across initial biochemical presentations even after excluding gender-specific causal agents. The findings support the above-mentioned hypothesis and also pose important clinical and pathophysiological questions relevant to gender-differences in DILI phenotypes, which should be further investigated across different disciplines.

Key points

- Liver biopsies obtained within 30 days of onset from 212 patients with drug-induced liver injury were analysed for the associations of gender and a proxy of female menopausal status with histological features in the acute injury phase.
- Biopsies of the 58 women <50 years of age were associated with more severe interface hepatitis and less iron-stained hepatocytes vs. those of the 87 men and 67 women ≥50 years.
- Compared to those of men, biopsies of women showed significantly greater plasma cell infiltration, hepatocyte apoptosis, hepatocyte rosettes and lobular disarray.
- Biopsies from men showed greater histological cholestasis.

2 | METHODS

2.1 | Study design and data source

This is a hypothesis-driven, cross-sectional analysis designed to investigate the associations of gender and female menopausal status with various histological features in patients with DILI. Data from the U.S. Drug-Induced Liver Injury Network (DILIN) were utilized in our analysis. The study design and data collection in the prospective DILIN database study have been previously described.[12] Briefly, the consortium enrols consecutive adult and paediatric patients with suspected DILI. Detailed clinical information, including laboratory data, medications, medical & social history, and symptoms and signs at DILI onset, was collected at the time of study enrolment. DILI onset was defined per protocol as date of initial presentation with elevated liver enzymes that met any of the study criteria.[12] Causality assessment was performed by the DILIN causality committee in a consensus manner using the DILIN causality score after 6 months follow-up. For subjects who showed evidence of persisting DILI,[13] additional follow-up evaluations were performed at 12 months and 24 months.[12] Liver biopsy was not required for study enrolment. When liver biopsy was performed for clinical indications, biopsy slides were obtained from the clinical study centres. The clinical decision on whether to perform a liver biopsy was solely made by the attending physician who often differed from the DILIN investigator. The DILIN studies were approved by the Institutional Review Board (IRB) at each participating centre (listed in Supplemental Methods). Informed consent was obtained from each of the participants prior to the study enrolment.

2.2 | Study population

Of 1386 enrolled patients between September 2004 and May 2014, 212 (15%) patients who met the following criteria were analysed: (i) Age >18 years (ii) an adequate, evaluable liver biopsy that had been performed within 30 days of DILI onset, (iii) a DILIN causality score of 'probable' or higher,[14] (iv) no prior diagnosis of other chronic liver diseases. The time window for this study was set to focus on histological features in acute phase.

2.3 | Study variables

2.3.1 | Predictors

The primary predictor variable tested in this study was the gender/female menopause classification. As reproductive information was not

collected as a part of the DILIN study, age 50 years old, the average age of female menopause in the US, was used as a surrogate.[15] Prior to our main analyses, we performed background analyses to assess an interaction between gender and age 50 in each histological feature to better characterize ageing effect vs. menopausal effect as detailed in Supplemental Statistical Method. Based on the background analyses, the study population was classified into three categories (hereafter called the gender/age categories), men, women younger than 50 years (surrogate for premenopausal), and women 50 years old or older (surrogate for postmenopausal) in our main analyses. Gender (men vs. all women) was also assessed as the secondary predictor.

2.3.2 | Outcomes

Liver biopsy slides stained with haematoxylin-eosin and Masson trichrome stain were obtained from the clinical study centres and were reviewed and scored in a blinded, standardized manner by a single experienced hepatopathologist [DEK].[16] Histological features, including interface hepatitis, plasma cell infiltration, cholestatic degree, hepatocellular cholestasis, canalicular cholestasis, hepatocyte rosettes, lobular disarray, iron stain-hepatocellular iron and apoptosis, were analysed as primary study outcomes in this study. Histological injury types were classified into the following two categories based on the histological injury patterns: cholestatic/mixed injury (ie, acute cholestatic, chronic cholestatic and combined hepatitic/cholestatic) and hepatocellular (ie, others).[16]

2.3.3 | Others

Initial biochemical injury types, hepatocellular [HC] injury and cholestatic/mixed [CS/MIX] injury, were determined based on the R ratio at the time of DILI onset or the closest to the onset when the laboratory data were not available at the onset, calculating the ratio of serum alanine aminotransferase (ALT)/upper limit normal (ULN) to serum alkaline phosphatase (ALP)/ULN: ≥5 (HC) vs. <5 (CS/MIX).[17] R-values were calculated using institutional reference ranges of serum ALT and ALP at the time of case enrolment. Information on demography, laboratory data at the study enrolment, and suspected drugs was also obtained for our analyses.

2.4 | Statistical analyses

Descriptive data are reported as mean±standard deviation or median with interquartile range for continuous variables and as a percentage for categorical variables. To assess selection bias because of the lack of liver biopsy within 30 days of DILI onset, clinical characteristics of the study population were compared between those included in the analysis vs. those not included in the analysis (ie, age >18 years, a DILIN causality score of 'probable' or higher, and no prior diagnosis of other chronic liver diseases, but no biopsies within 30 days of DILI onset).

Clinical characteristics of the study population were compared among the gender/age categories by using Kruskal–Wallis test for continuous and ordinal variables and chi-square test for categorical variables. Histological features of the study population were similarly compared among the gender/age categories with stratification on different biochemical injury types.

For modelling the histological features, univariate analysis was performed first to select potential clinical covariates for adjustment in the multivariate models. To determine a proper age variable to be included in a model, background analyses were performed as described in Supplemental Statistical Methods. Depending on the histological outcomes (binary vs. ordinal), logistic regression models or ordinal logistic regression models were used. For the ordinal logistic regression, proportional odds models or cumulative logistic regression models were selected, depending on whether or not the proportional odds assumption was met for a specific histological outcome. Adjusted odds ratio or cumulative odds ratio with 95% confidence interval (CI) and *P* value were reported. The fit of the models was assessed with Hosmer-Lemeshow goodness-of-fit test for binary and ordinary outcomes.[18]

Statistical analyses were performed using SAS version 9.4. (SAS Institute, Cary, NC). All *P* values presented are two-sided, and the differences were considered statistically significant when the $P<.05$. For detecting interactions in the background analyses, we used $P<.1$ because of the small sample size. Because of the descriptive nature of this analysis, *P* values have not been adjusted for multiple comparisons.

3 | RESULTS

3.1 | Clinical characteristics

A total of 212 adults with DILI were included in the analysis. Clinical characteristics of the study population at enrolment are summarized in Table 1. Women of age <50 years, women of age ≥50 years and men comprised 27.4%, 31.6% and 41.0% of the study population respectively. Mean [±SD] age of the total study population was 50±16 years old. Seventy-seven percentage were white, 14.6% were black and 10% were Hispanic. Fifty percentage of the cases presented as HC injury at DILI onset. Age, the biochemical presentation at DILI onset, serum ALT, serum AST, serum ALP and positive ANA were significantly different among the gender/age categories (Table 1). Hepatocellular injury was noted in 70.7% of women of <50 years compared to 43.3% of women of ≥50 years and 41.4% of men. Serum ALT and AST were the highest in women aged <50 years, while serum ALP was the highest in women aged ≥50 years. Men had a lower frequency of positive ANA (14.5%) compared to women (>30% in both groups) at the study enrolment. Black, other race and Hispanic were more prevalent among women of age <50 years although there were no statistical significances.

The clinical characteristics of the study population were then compared with the patients excluded from this analysis because of the lack of liver biopsy within 30 days of the DILI onset. The clinical characteristics of the study population (N=212) vs. the population not included in this analysis (N=792) are summarized in Table S2. Briefly, clinical severity score, serum ALP, and total bilirubin at DILI onset were higher in the study population. The age, the gender/age categories,

TABLE 1 Clinical Characteristics of the study population

Clinical characteristics	Total N=212	Women <50 N=58	Women ≥50 N=67	Men N=87	*P*-value
Age, year, mean±SD	50.1±15.7	37.1±9.3	62.4±9.0	49.4±15.9	<.001
Race					
White, %	77.4	63.8	80.6	83.9	.077
Black, %	14.6	24.1	11.9	10.3	
Others, %	8.0	12.1	7.5	5.7	
Ethnicity Hispanic, %	10.0	15.5	9.1	6.9	.242
Biochemical injury type: Hepatocellular injury*, %	50.0	70.7	43.3	41.4	.002
Liver chemistries at onset, median(25^{th}, 75^{th})					
ALT, U/L	538 (245, 1268)	770 (267, 1522)	649 (331, 1193)	418 (173, 948)	.011
AST, U/L	353 (137, 989)	738 (224, 1437)	557 (162, 1092)	205 (86, 553)	<.001
ALP, U/L	245 (161, 385)	215 (153, 306)	315 (220, 426)	225 (138, 387)	<.001
Total bilirubin, mg/dL	7.3 (3.8, 11.9)	5.8 (3.3, 10.4)	6.6 (3.4, 11.9)	8.5 (5.0, 12.9)	.054
INR	1.2 (1.0, 1.5)	1.2 (1.1, 1.6)	1.2 (1.0, 1.7)	1.1 (1.0, 1.3)	.336
Positive ANA, %	26.7	30.4	38.8	14.5	.002
Time from DILI onset to Liver biopsy, days	9 [5, 15]	7 [5, 13]	9 [5, 15]	9 [4, 15]	.4671
Primary implicated agents, N**					
Herbs-Dietary Supplements#	53	10	11	32	
Amoxicillin/clavulanic acid	26	2	7	17	
Minocycline	8	4	2	2	
Nitrofurantoin	6	1	5	0	
Cefazolin	6	2	3	1	
Ciprofloxacin	5	1	3	1	
Levofloxacin	4	1	1	2	
Allopurinol	4	0	2	2	

*: *R*-value ≥5. **: Distributions of implicated drugs (≥4) are provided above. #: Of them, eleven cases were caused by anabolic steroids.

self-reported race/ethnicity, biochemical injury type at DILI onset, and positive ANA did not show statistical differences between the populations. Biochemical injury type, clinical severity score and positive ANA were further analysed, classifying by the age/gender categories in each population (Table S3). No statistically significant interactions (the populations x the age/gender categories) were noted.

Causal agents implicated in the study population are provided in Table S1a,b. Among agents implicated in ≥4 cases (Table 1), Herbs-Dietary Supplement (HDS) was most prevalent (25%), followed by amoxicillin/clavulanic acid (12.2%). Eleven cases of the HDS-related DILI were caused by anabolic steroids, all of whom were males.

3.2 | Univariate associations of the histological features with the gender/age categories

The previous analysis demonstrated the histological features significantly differ depending on the initial biochemical presentations: hepatocellular vs. cholestatic/mixed injury.[16] Therefore, the univariate associations were assessed not only in the total study population but also within the groups of hepatocellular injury and cholestatic/mixed injury separately (Table 2). Several variables were significantly associated with the gender/age categories. It is notable that, across the injury types, interface hepatitis, noticeable increase in plasma cells, apoptosis, hepatocyte rosettes and lobular disarray showed a female-dominant pattern while cholestasis and hepatocyte iron-positivity by Perls' staining showed a male-dominant pattern. These histological features were considered in the multivariable analyses.

3.3 | Adjusted associations of the histological features with the gender/age categories

In our background analyses, a potential effect of advancing age was only evident in interface hepatitis at age of 70 in both men and women. The age category (<50 vs. ≥50) did not show significant associations with any histological features in men. There was an interaction of gender and the age category in the histological features of interface hepatitis (*P*=.05) and apoptosis (*P*=.06).

The adjusted associations of histological features with (i) the gender/age categories (Model 1) and (ii) gender (Model 2) are presented in Tables 3,4. All the models were adjusted for biochemical injury types,

TABLE 2 Univariate associations of the gender/age group categories with histological features

	Total population				Hepatocellular injury				Cholestatic/mixed injury			
	F <50	F ≥50	Men		F <50	F ≥50	Men		F <50	F ≥50	Men	
	N=58	N=67	N=87		N=41	N=29	N=36		N=17	N=38	N=51	
Interface Hepatitis, %												
Grade 0	3.6	4.5	10.6	**	0.0	0.0	11.8	*	11.8	8.1	9.8	
Grade 1	19.6	40.9	35.3		12.8	20.7	20.6		35.3	56.8	45.1	
Grade 2	10.7	16.7	25.9		10.3	13.8	26.5		11.8	18.9	25.5	
Grade 3	26.8	13.6	14.1		30.8	17.2	11.8		17.6	10.8	15.7	
Grade 4	39.3	24.2	14.1		46.2	48.3	29.4		23.5	5.4	3.9	
Noticeable plasma cell infiltration, %	37.5	31.8	11.6	**	41.0	53.6	14.3	*	29.4	15.8	9.8	
Cholestasis, degree, %												
Grade 0	48.2	46.3	19.8	**	53.8	58.6	31.4	*	35.3	36.8	11.8	**
Grade 1	17.9	16.4	14.0		23.1	24.1	22.9		5.9	10.5	7.8	
Grade 2	26.8	20.9	27.9		17.9	10.3	34.3		47.1	28.9	23.5	
Grade 3	7.1	16.4	38.4		5.1	6.9	11.4		11.8	23.7	56.9	
Hepatocellular cholestasis, %	41.1	47.8	72.1	**	30.8	31.0	57.1	*	64.7	60.5	82.4	
Canalicular cholestasis, %	50.0	52.2	74.4	**	43.6	41.4	62.9		64.7	60.5	82.4	
Hepatocyte rosettes (more than rare), %	51.8	30.3	17.6	**	61.5	53.6	38.2		29.4	13.2	3.9	*
Lobular disarray present, %	42.9	24.2	15.1	**	53.8	46.4	34.3		17.6	7.9	2.0	*
Iron-stain-hepatocytes, %												
Grade 0	81.5	63.9	35.9	**	81.6	65.4	46.7	*	81.3	62.9	29.2	**
Grade 1 -2	18.5	36.1	64.1		18.4	34.6	53.3		18.8	37.1	70.8	
Apoptosis, %												
Grade 0	10.7	17.9	22.4	**	5.1	6.9	11.8		23.5	26.3	29.4	
Grade 1	33.9	47.8	61.2		28.2	31.0	47.1		47.1	60.5	70.6	
Grade 2	55.4	34.3	16.5		66.7	62.1	41.2		29.4	13.2	0.0	
Lobular inflammation, %												
Grade 0	0.0	0.0	1.2		0.0	0.0	2.9	*				
Grade 1	10.7	10.4	11.6		2.6	10.3	11.4		29.4	10.5	11.8	
Grade 2	5.4	17.9	17.4		2.6	10.3	11.4		11.8	23.7	21.6	
Grade 3	17.9	25.4	23.3		12.8	6.9	17.1		29.4	39.5	27.5	
Grade 4	66.1	46.3	46.5		82.1	72.4	57.1		29.4	26.3	39.2	
Lymphoid aggregates/Germinal centres, %	3.6	12.1	4.7		2.6	25.0	8.6	*	5.9	2.6	2.0	
PAS-positive macrophage, %												
Scattered	13.2	21.7	29.7		5.4	28.0	24.1	*	31.3	17.1	33.3	
Clusters	86.8	78.3	70.3		94.6	72.0	75.9		68.8	82.9	66.7	
Lipogranulomas present,%	5.4	21.2	17.4	*	7.7	3.6	8.6		0.0	34.2	23.5	*
Copper-stained hepatocytes, %												
Grade 0	92.6	91.9	82.9		89.7	92.6	93.1		100.0	91.4	76.6	*
Grade 1	7.4	8.1	15.8		10.3	7.4	6.9		0.0	8.6	21.3	
Grade 2	0.0	0.0	1.3		0.0	0.0	0.0		0.0	0.0	2.1	

Histological grades were scored in a standardized manner.16 *P<.05, **P<.001: for the comparison among gender/age categories (Kruskal–Wallis or Chi-square test). Eosinophil infiltration, neutrophil infiltration, central vein endophlebitis, nodular transformation, confluent necrosis, hepatocyte ballooning, cholangiolar cholestasis, ductular reaction and sinusoidal reticuloendothelial iron stain were not significantly associated with the gender/age categories in any of the above analyses (data not shown).

TABLE 3 Associations of the gender/age group categories with histological features using logistic and ordinal logistic regression models

	Model 1				Model 2	
	Men vs. Women<50(ref)		Women≥50 vs. Women<50(ref)		Men vs. Women(ref)	
Histological features	Odds ratio (95% CI)	*P* value	Odds ratio (95% CI)	*P* value	Odds ratio (95% CI)	*P* value
Interface hepatitis[a,*]	0.42 (0.22, 0.81)	.009	0.51 (0.26, 1.02)	.055	0.63 (0.37, 1.05)	.076
Noticeable plasma cell infiltration[b]	0.30 (0.13, 0.74)	.009	1.15 (0.50, 2.50)	.792	0.29 (0.13, 0.62)	.002
Cholestasis degree[b,*]	3.63 (1.82, 7.27)	<.001	0.94 (0.46, 1.92)	.868	3.76 (2.15, 6.57)	<.001
Hepatocellular cholestasis[b]	2.85 (1.33, 6.08)	.007	0.94 (0.43, 2.04)	.876	2.95 (1.58, 5.48)	<.001
Hepatocyte rosettes[b]	0.29 (0.12, 0.66)	.004	0.62 (0.27, 1.43)	.260	0.37 (0.18, 0.76)	.007
Canalicular cholestasis[b]	2.36 (1.12, 4.99)	.025	0.87 (0.41, 1.84)	.712	2.56 (1.38, 4.73)	.003
Lobular disarray[b]	0.36 (0.15, 0.87)	.023	0.69 (0.29, 1.66)	.407	0.43 (0.20, 0.94)	.033
Iron Satin-hepatocellular iron[b]	8.07 (3.33, 19.53)	<.001	2.53 (1.02, 6.28)	.046	4.53 (2.41, 8.54)	<.001

[a]adjusted for liver injury type, black race and age≥70; [b]adjusted for liver injury type, black race.
Model 1 was to assess the associations of histological features in men and women ≥50 vs. women <50 (reference) while Model 2 was to assess the associations of histological features in men vs. women (reference). Logistic regression models were used except for the two ordinal variables (*) where proportional odds models were used. For the two ordinal variables, proportional odds assumption was met (score tests). The variable of cholestatsis degree was analysed with three ordinal categories of Grade 0, 1-2, and 3 because of better model fitting. The odds ratio presented is either the usual odds ratio or cumulative odds ratio with odds of higher level categories vs. lower level categories. Therefore, an odds ratio of >1 represents an increased likelihood of having a worse outcome.

TABLE 4 Associations of the gender/age group categories with histological severity of apoptosis using a cumulative logistic regression model

	Model 1		Model 2
Apoptosis	Men vs. Women<50 COR (95% CI), *P* value	Women ≥50 vs. Women<50 COR (95% CI), *P* value	Men vs. Women COR (95% CI), *P* value
Grade 1-2 vs. Grade 0	0.57 (0.19, 1.67), 0.303	0.73 (0.24, 2.20), 0.577	0.70 (0.33, 1.50), 0.359
Grade 2 vs. Grade 0-1	0.25 (0.11, 0.60), 0.002	0.76 (0.33, 1.76), 0.523	0.29 (0.14, 0.62), 0.001

Categories of Apoptosis: Grade 0=none to rare, Grade 1=mild, Grade 2=moderate. COR: cumulative odds ratio. Cumulative logistic regression model was fit to the ordinal variable of apoptosis because the proportional odds assumption was not satisfied. The models were adjusted for biochemical injury type and black race with women of <50 as a reference group (Model 1) or women as a reference group (Model 2). The cumulative odds ratio was computed as an odds of higher level categories vs. lower level categories, therefore, an odds ratio of >1 represents an increased likelihood of having a worse outcome.

black race, and, for interface hepatitis only, the age category (<70 vs. ≥70). The goodness of fit tests indicated an adequate fit (*P*-values of .27-.99). More severe interface hepatitis and less hepatocyte iron stains were noted in biopsies of women <50 years compared to those of men and women ≥50 years (*P*=.009 and *P*=.055 for interface hepatitis and *P*<.001 and *P*=.046 for hepatocyte iron stains respectively). Compared to those of men, biopsies of women were associated with more plasma cell infiltration, more apoptosis, more hepatocyte rosettes, more lobular disarray, and less iron-stained hepatocytes and less cholestasis (*P*<.05).

Amoxicillin/clavulanic acid, anabolic steroids and nitrofurantoin, which showed gender-specific distributions in this study population, have been associated with signature injury patterns. Therefore, we assessed the associations between the histological features and the gender/age categories (or gender) after excluding these agents. These associations remained similar even after excluding cases because of anabolic steroids, nitrofurantoin or amoxicillin/clavulanic acid (data are not shown).

The associations of histological features with the gender/age categories and gender were also assessed using the same modelling strategy but adjusting for the histological injury classification, which showed similar results, except for hepatocellular/canalicular cholestasis; the odds ratios of men vs. women were decreased for hepatocellular/canalicular cholestasis (data are not shown).

4 | DISCUSSION

This hypothesis-refining analysis revealed intriguing findings pertaining to gender-specific phenotypes of DILI. Women <50 years of age were more likely to show severe interface hepatitis and less hepatocyte iron staining compared to men and women ≥50 years. The comparable risk reduction in women ≥50 years and men vs. women <50 years of age implies the involvement of female sex hormones as opposed to innate sex difference in the pathology involved in these features. For this interpretation, we considered a possible indication bias; young women with positive ANA and high serum ALT and AST might have undergone liver biopsies more often than others to rule out autoimmune hepatitis. However, we found no evidence of bias

regarding frequencies of liver biopsies that could explain more severe autoimmune hepatitis features in women aged <50 vs. women aged ≥50 and men (Tables S2,S3); the study population appeared to have similar proportions of positive ANA in each age/gender category as compared to the excluded population (interaction *P*=.29). Positive ANA is observed among healthy general population.[19,20] Thus whether positive ANA is an innocent bystander, or a risk factor of developing hepatotoxicity, or a consequence of hepatotoxicity observed in a subgroup of patients is uncertain. Numbers of iron-positive hepatocytes were the highest in men, intermediate in women ≥50 years, and the lowest in women <50 years of age. Similar male dominance in hepatic iron deposition has been reported in a cohort of NAFLD.[21] This might be explained by blood loss of menstruation, pregnancy, and breast feeding and/or oestrogen's effect on hepcidin.[22]

Men had a higher likelihood of cholestatic features with a higher severity compared to women. Of note, our 'risk of bias' assessment showed comparable biochemical injury type within the age/gender categories between the study population and the excluded population (interaction *P*=.67 in Table S3). The consistent observation after excluding the cases caused by anabolic steroids or amoxicillin/clavulanic acid ruled out the possible explanation by male dominance observed with these agents (data not shown). Unlike the autoimmune hepatitis features, the gender difference in cholestatic features appeared to be consistent before and after the age 50 years. Our extended analysis showed that male gender (OR and 95%CI=2.0 [1.1, 3.6], *P*=.019) and age ≥50 years (OR and 95%CI=3.0 [1.7, 5.4], *P*=.0001) were independently associated with the cholestatic/mixed injury (R≤2). This observation is consistent with previous observational studies.[3,23] Although underlying mechanisms are uncertain, these findings suggest a gender difference in cholestasis, independent of age and female sex hormones. The models adjusting for the histological injury classification showed consistent associations except for hepatocellular/canalicular cholestasis. Hepatocellular/canalicular cholestasis could develop in severe hepatocellular injury as a consequence of compromised cellular energy supply and subsequent impairment of highly energy-dependent transporters such as BSEP,[24] which are difficult to distinguish from true cholestatic injury without considering overall histological pictures.

It is well-known that women are more prone to develop autoimmune disorders of all types than are men and that sex hormones significantly modulate innate as well as adaptive immune responses.[7] Women in general induce stronger antibody-production and cell-mediated immune responses following either infection or vaccination than men.[7] Also, there are quite a few experimental studies showing how oestrogens and progesterone influence cellular stress response and severity of liver injury. Oestrogens are in general reported to be protective against liver injury[25,26] while progesterone appears to exert detrimental impacts on inflammation and fibrosis.[27,28] Thus, impact of sex and sex hormones on host response to drug toxicity is likely multilayered[29] and modulates gender-specific clinical phenotypes.

Another noteworthy finding in our analysis was that black race was associated with about two-fold higher likelihood of having more noticeable plasma cell infiltration across the different models (*P*-value of .048 to .08; data are not shown). This is intriguing as black race is reportedly associated with an enhanced humoral immunological response following vaccination compared to other races.[30] A previous study reported that black women were overrepresented among non-acetaminophen induced acute liver failure.[31,32] As women were associated with more noticeable plasma cell infiltration, there may be an additive interaction between gender and genetic predisposition causing an enhanced humoral immune response in DILI susceptibility and DILI severity.

A strength of this study is that the analysis was performed using a well-characterized DILI population with careful follow-up for up to 2 years in which data were collected and evaluated in a standardized manner.[14,33] Also, we applied different modelling approaches to enhance our data interpretation and theory-generation. This study also has several limitations. We used a proxy of menopause and the information on exact menopausal state was not available. Possible misclassification among women may have blunted the true associations. Sex hormone levels were not obtained. Our study population may have been biased because of the requirement of liver biopsy data. Although we evaluated the 'risk of bias' and discussed it above, there may be a bias unmeasured in this study. Therefore, our findings may be applicable only to those who developed clinically significant drug-induced liver injury and who are likely considered for liver biopsy. The initiation of liver injury and the type of initial presentation are likely a function of drug and host and may be determined by their specific interplay.[29] Host–drug interplay in DILI is beyond the scope of this analysis. Our analysis was meant to address the impact of gender and menopause on 'host response' to DILI 'after' the initiation of injury, but not impact on the initiation of liver injury or tissue recovery. Host response to drug toxicity is likely modified by multiple host factors, including concomitant medications and pre-existing co-morbidities,[29] which were not analysed in this analysis. Race/ethnicity, other than Caucasian or black, also could not be included in this analysis because of their low frequencies. Lastly, our analytic strategy was built based on our hypothesis. Whether our analytic approach is justifiable from a mechanistic viewpoint may be questioned. We separately developed a model considering all the available variables at study enrolment, independent of the hypothesis. This model also identified gender as a significant contributor in the histological features analysed in this study, which further supports the significance of gender in DILI histology.

In summary, our analysis demonstrated that histological features of DILI were significantly associated with gender and age [used as a proxy of menopause] in patients presenting with varying laboratory profiles. The findings support our hypothesis and generated several additional intriguing hypotheses relevant to gender differences in injury/stress responses to drug toxicity (eg, cell death pathway, inflammation and immune response). Further clinical analyses and the translation to experimental studies are warranted to delineate gender-dependent hepatotoxicity and DILI manifestations.

CONFLICT OF INTEREST

The authors have the following disclosures: Ayako Suzuki: served as a consultant for GlaxoSmithKline; Herbert L. Bonkovsky: served

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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