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Patient Sex, Reproductive Status, and Synthetic Hormone use Associate With Histologic Severity of Nonalcoholic Steatohepatitis

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Abstract

Sex and sex hormones can affect responses of patients with nonalcoholic fatty liver disease (NAFLD) to metabolic stress and development of hepatocyte injury and inflammation. We

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collected data from 3 large US studies of patients with NAFLD (between October 2004 and June 2013) to assess the association between histologic severity and sex, menopause status, synthetic hormone use, and menstrual abnormalities in 1112 patients with a histologic diagnosis of NAFLD. We performed logistic or ordinal logistic regression models, adjusting for covariates relevant to an increase of hepatic metabolic stress. We found that pre-menopausal women were at an increased risk of lobular inflammation, hepatocyte ballooning, and Mallory-Denk bodies than men and also at an increased risk of lobular inflammation and Mallory-Denk bodies than post-menopausal women ($P<.01$). Use of oral contraceptives was associated with an increased risk of lobular inflammation and Mallory-Denk bodies in pre-menopausal women, whereas hormone replacement therapy was associated with an increased risk of lobular inflammation in post-menopausal women ($P<.05$). We conclude that being a pre-menopausal woman or a female user of synthetic hormones is associated with increased histologic severity of hepatocyte injury and inflammation among patients with NAFLD at given levels of hepatic metabolic stress.

Keywords

gender; differences; fibrosis; risk factor analysis

Introduction

Gender differences exist in nonalcoholic fatty liver disease (NAFLD). Women are at a decreased risk of NAFLD compared to men at their reproductive age while after menopause women lose the protective effect.¹⁻³ The risk of NAFLD in post-menopausal women appears diminished by the use of hormone replacement therapy (HRT).⁴ The data suggest protective effects of female sex hormones against NAFLD. Among adult patients with nonalcoholic steatohepatitis (NASH), the risk of hepatic fibrosis differs based on gender and menopausal state; pre-menopausal women have a decreased likelihood of hepatic fibrosis compared to men and post-menopausal women for a given grade of portal inflammation and hepatocyte ballooning.^{5, 6} This suggests that at the same level of fibrogenic stimuli (i.e., hepatocyte injury and inflammation), men and post-menopausal women are at a higher risk of hepatic fibrosis compared to pre-menopausal women.

How gender and menopause modulate host response to metabolic stress and influence the severity of hepatocyte injury and inflammation in NAFLD remains unknown. This is important as fibrogenesis is mainly driven by fibrogenic stimuli generated via hepatocyte injury and inflammation.^{7, 8} Further, most of current and future potential therapies target the reduction of metabolic stress, hepatocyte damage, and/or inflammation. Thus, better understanding of gender differences in such mechanisms is crucial to personalizing our therapeutic approach. We hypothesize that, in adults, gender and different physiological sex hormone levels modulate hepatic injury and inflammation in response to metabolic stress in patients with NAFLD. In this study, we investigated the relationships of NASH histologic severity to gender, reproductive status, and hormone therapies at a given level of metabolic stress. We also assessed the associations of the histologic features to women-specific reproductive variances (menstrual abnormality, oral contraceptive use, and HRT) in pre- and post-menopausal women.

Patients and Methods

Study design and Population

This is a hypothesis-driven, cross-sectional study. Data from the NASH Clinical Research Network (CRN) Database (DB) study, the Pioglitazone versus Vitamin E versus Placebo for the Treatment of Non-diabetic Patients with Nonalcoholic Steatohepatitis (PIVENS) trial at baseline, and NASH CRN Adult Database 2 project (DB2, before November, 2013) were utilized.⁹⁻¹¹

Among adult patients (age ≥ 18 years) who had liver biopsy within 6 months of enrollment (N=1250), subjects not meeting histologic criteria for NAFLD (N=48) or reporting excess alcohol intake [≥ 7 (women) or ≥ 14 (men) servings per week] (N=2) were excluded. Pre-menopausal women with age 45 or older (N=80), pre-menopausal women who reported estrogen-only product use (N=3), and post-menopausal women who reported progesterone-only product use (N=5) were removed from the analysis due to concerns over peri-menopausal state or potential misclassification of menopausal state. The NASH CRN studies were approved by the Institutional Review Boards at all sites.

Liver histology

All liver biopsy specimens were reviewed and scored by the NASH CRN Pathology Committee in a consensus manner according to the NASH CRN scoring system.⁹

Study variables

Variables of menopausal state, menstrual patterns, and synthetic hormone use were created based on self-reported information obtained via the study questionnaires.⁹⁻¹¹ The gender/menopause classification (men, pre-menopausal women, and post-menopausal women), menstrual patterns (none to rare vs. others), oral contraceptive use, and HRT use were assessed for the associations with histologic grades of lobular inflammation, hepatocyte ballooning, portal inflammation and Mallory-Denk bodies.

Covariates for the models were selected based on key mechanistic concepts related to an increased hepatic metabolic stress, an increased free fatty acid delivery to the liver and cellular senescence¹²: age (years), waist circumference [cm] standardized by body mass index [(BMI) (kg/m²)¹², the homeostatic model assessment of insulin resistance (HOMA-IR)¹³, and steatosis grade. Demographic data, alcohol consumption (average servings per week), smoking history (current smokers vs. others), self-reported diagnosis of polycystic ovary syndrome (PCOS), hypertension, and diabetes/impaired glucose tolerance were also collected at the study enrollment and considered in the analysis.⁹⁻¹¹

Statistical analyses

Clinical and histologic characteristics of the study population were compared among the gender/menopause categories using analysis of variance (ANOVA) or Kruskal-Wallis test for continuous variables and chi-square test for categorical variables. Histologic scores were compared using Kruskal-Wallis tests with Wilcoxon rank-sum tests as post-hoc pairwise comparisons.

Multiple logistic (or ordinal logistic) regression model (MLR or MOLR) were used to assess the associations between the gender/menopause categories and severity of NASH histologic features while adjusting for the above-mentioned covariates, with and without including other potential confounding factors. The associations of menstrual abnormality, oral contraceptive use and HRT to the histologic features were also assessed in pre- or post-menopausal women using the same modeling approach. Significance of the associations was determined using likelihood ratio tests. Adjusted odds ratios (AOR) or cumulative odds ratios (ACOR) with 95% confidence interval (CI) were computed using the models.

All the statistical analyses were performed using JMP statistical software v.9.0 (SAS Institute, Cary, NC).

Results

Patient characteristics

Men, pre-menopausal women, and post-menopausal women comprised 42%, 14%, and 44% of the study population (N=1112). Clinical characteristics and histologic features of NAFLD are summarized for the gender/menopause categories in Table 1.

Associations of gender, reproductive status, and synthetic hormones with NASH histologic features

The associations of gender and menopause with NASH histologic features are summarized in Table 2. Compared to pre-menopausal women, men had a decreased risk of having severe lobular inflammation, hepatocyte ballooning and Mallory-Denk bodies while post-menopausal women had a decreased risk of having severe lobular inflammation and Mallory-Denk bodies (Model 1, Table 2). The results remained consistent even after adjusting for other potential clinical confounders (Model 2, Table 2).

Oral contraceptive use in pre-menopausal women was associated with an increased likelihood of having more severe lobular inflammation and Mallory-Denk bodies (Supplementary table 1, Model 1). HRT in post-menopausal women was associated with an increased risk of having severe lobular inflammation (Supplementary Table 2, Model 1,2).

PCOS was not associated with NASH histologic features in both univariate/multivariate analyses and association between oral contraceptive and NASH histologic features were not altered after adjusting for PCOS (Data not shown).

Discussion

Our analysis showed that pre-menopausal women have a higher grade of lobular inflammation, hepatocyte ballooning and Mallory-Denk bodies compared to men at a given level of metabolic stress. In the sub-analyses of women only, oral contraceptive use was associated with an increased risk of having more severe lobular inflammation and Mallory-Denk bodies among pre-menopausal women. In post-menopausal women, HRT was associated with an increased risk of having more severe lobular inflammation.

The above findings suggest that gender, menopause and synthetic hormone use may modulate degree of hepatocyte injury and inflammation in response to metabolic stress and influence the intensity of fibrogenic stimuli which drive fibrogenesis. Histologic grades of lobular inflammation and Mallory-Denk bodies were higher in pre-menopausal women vs. men and post-menopausal women, suggesting hormonal effects. This is counterintuitive as most of the data from animal models show 'protective' effects of estrogens on liver injury, regardless of injury stimuli.¹⁴⁻¹⁵ Our extended analysis in which variables of progesterone use and estrogen use were separately incorporated in the model, progesterone use, but not estrogen use, was associated with more severe lobular inflammation and Mallory-Denk bodies (data not shown). This suggests the detrimental impact may be explained by a higher level of progesterone, but not estrogen. Further investigation on how gender and sex hormone levels differentially influence NAFLD pathobiology, cellular stress response, and cell death is warranted.

It is noteworthy that, despite their enhanced hepatocyte injury and inflammation, pre-menopausal women were associated with a lower stage of hepatic fibrosis compared to post-menopausal women and men. Further, consistent with the previous studies⁵⁻⁶, pre-menopausal women were at a decreased risk of hepatic fibrosis compared to men and postmenopausal women at a given level of hepatocyte ballooning and portal inflammation in this independent population (data not shown). Thus, impacts of gender/sex hormones on NASH features appear to be multifaceted. Usage of oral contraceptive in women who have multiple NAFLD risk factors could be detrimental, especially in women who have a condition of estrogen depletion. Further validation studies are required to develop robust clinical recommendations.

This study has several limitations including potential referral bias (patients were enrolled at tertiary academic centers), recall bias (self-reported menopausal category and synthetic hormone use), the lack of information on cumulative estrogen and/or progesterone exposure (e.g., pregnancies, duration of synthetic hormone use), and possible under diagnosis of PCOS. Due to the cross-sectional nature, duration of exposure to an increased metabolic stress in the liver was not taken into account in the analysis. Lastly, we did not measure serum estrogen/progesterone levels or their signaling pathway activation. Therefore, whether the observed associations can be explained by hormonal effects versus innate sex differences cannot be confirmed. Nonetheless, our analysis provides hypotheses pertaining to gender differences and multifaceted impact of sex hormones in NAFLD, which can be tested in future multi-disciplinary investigations.

In conclusion, our hypothesis-driven analysis demonstrated distinct associations of gender, menopause, oral contraceptive use and HRT with hepatic lobular inflammation/injury, providing an interesting theory pertaining to complex effects of sex and sex hormones on human NAFLD. Gender-differences in NASH clinical phenotypes and pathophysiology should be further characterized in future studies to develop a personalized approach to patients with NAFLD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

ACOR	adjusted cumulative odds ratio
BMI	body mass index
COR	cumulative odds ratio
CI	confidence interval

HRT	hormone replacement therapy
MOLR	multiple ordinal logistic regression model
NAFLD	non-alcoholic fatty liver disease
NASH	non-alcoholic steatohepatitis
NASH CRN	NASH Clinical Research Network

Table 1

Clinical and Histologic Characteristics of the Study Population

	Pre-menopausal women (N=160)	Post-menopausal women (N=489)	Men (N=463)	P value
Age (years)	34.4 ± 7.3	57.6 ± 7.4	45.8 ± 12.2	<0.01
Race/Ethnicity				<0.01
White	103 (64.4 %)	376 (77.2 %)	345 (74.8 %)	
Black	6 (3.8 %)	20 (4.1 %)	12 (2.6 %)	
Others	14 (8.8 %)	48 (9.9 %)	52 (11.3 %)	
Hispanic	37 (23.1 %)	43 (8.8 %)	52 (11.3 %)	
BMI (kg/m²)	37.3 ± 7.8	34.4 ± 6.6	33.8 ± 5.8	<0.01
Waist (cm)	112.0 ± 15.8	106.9 ± 13.9	112.1 ± 14.2	<0.01
Diabetes/impaired glucose tolerance, %	71 (44.4%)	293 (59.9%)	224 (48.4%)	<0.01
HOMA-IR*	4.3 [2.7-7.2]	4.8 [2.8-8.6]	4.4 [2.7-6.9]	0.11
Hypertension, %	52 (32.5%)	364 (74.4%)	265 (57.2%)	<0.01
Alcohol consumption#	94 (58.8%)	196 (40.1%)	269 (58.1%)	<0.01
Current smoking	17 (10.6%)	47 (9.6%)	32 (6.9%)	0.20
Steatosis				<0.01
Grade 0	2 (1.3%)	21 (4.3 %)	14 (3.0%)	
Grade 1	47 (29.4%)	203 (41.5 %)	172 (37.2 %)	
Grade 2	60 (37.5%)	152 (31.1 %)	181 (39.1 %)	
Grade 3	51 (31.9%)	113 (23.1 %)	96 (20.7 %)	
Lobular inflammation				<0.01
Grade 0	0 (0%)	1 (0.2%)	4 (0.9%)	
Grade 1	66 (41.3%)	271 (55.4%)	272 (58.8%)	
Grade 2	69 (43.1%)	163 (33.3%)	162 (35.0%)	
Grade 3	25 (15.6%)	54 (11.0%)	25 (5.4%)	
Hepatocyte ballooning				<0.01
Grade 0	48 (30.0%)	141 (28.8 %)	184 (39.7 %)	
Grade 1	48 (30.0%)	122(25.0 %)	151 (32.6 %)	

	Pre-menopausal women (N=160)	Post-menopausal women (N=489)	Men (N=463)	P value
Grade 2	64 (40.0%)	226 (46.2 %)	128 (27.7 %)	
Mallory-Denk bodies				<0.01
No	111 (69.4%)	303 (62.0%)	373 (80.6%)	
Yes	49 (30.6 %)	186 (38.0%)	90 (19.4%)	
Portal inflammation				<0.01
Grade 0	23 (14.4%)	46 (9.4%)	68 (14.7%)	
Grade 1	119 (74.4%)	293 (60.0%)	298 (64.4%)	
Grade 2	18 (11.3%)	149 (30.5%)	97 (21.0%)	
Fibrosis				<0.01
Stage 0	46 (28.8%)	106 (21.7%)	117 (25.3%)	
Stage 1	67 (41.9%)	118 (24.2%)	139 (30.0%)	
Stage 2	27 (16.9%)	95 (19.5%)	95 (20.5 %)	
Stage 3	17 (10.6%)	114 (23.4%)	73 (15.8 %)	
Stage 4	3 (1.9%)	55 (11.3%)	39 (8.4 %)	
NAFLD diagnosis				<0.01
Steatosis without inflammation	29 (18.1%)	94 (19.2%)	113 (24.4%)	
Borderline NASH zone 3	28 (17.5%)	85 (17.4%)	116 (25.1%)	
Borderline NASH zone 1	4 (2.5%)	6 (1.2%)	4 (0.9%)	
Definite NASH	99 (61.9%)	304 (62.2%)	230 (49.7%)	

* Median [Interquartile range]

Self-reported alcohol consumption of <7 servings per week (women) and < 14 servings per week (men). In pairwise comparisons of the histologic features, grade of steatosis and lobular inflammation were higher in pre-menopausal women compared to men ($p<0.01$) and post-menopausal women ($p<0.01$). Grade of hepatocyte ballooning and the presence of Mallory-Denk bodies were lower in men compared to pre- ($p<0.01$) and post- ($p<0.01$) menopausal women. Stage of liver fibrosis and grade of portal inflammation were the highest in post-menopausal women, followed by men and pre-menopausal women ($p<0.01$).

Table 2

Gender/Menopause and pathologic features of NAFLD

	Univariate		Model 1		Model 2	
	COR[95%CI]	P value	ACOR[95%CI]	P value	ACOR[95%CI]	P value
Lobular inflammation						
Gender/menopause categories		<0.01		<0.01		<0.01
Pre-menopause	-				-	
Post-menopause	0.57 [0.41-0.81]		0.54 [0.34-0.85]		0.53 [0.34-0.84]	
Men	0.45 [0.32-0.63]		0.41 [0.28-0.61]		0.41 [0.28-0.61]	
Hepatocyte ballooning						
Gender/menopause categories		<0.01		<0.01		<0.01
Pre-menopause	-				-	
Post-menopause	1.19 [0.86-1.66]		0.78 [0.51-1.21]		0.74 [0.48-1.15]	
Men	0.62 [0.44-0.86]		0.55 [0.38-0.80]		0.54 [0.37-0.79]	
Mallory-Denk bodies						
Gender/menopause categories		<0.01		<0.01		<0.01
Pre-menopause	-				-	
Post-menopause	1.39 [0.95-2.05]		0.51 [0.30-0.87]		0.47 [0.27-0.81]	
Men	0.55 [0.36-0.82]		0.33 [0.20-0.54]		0.30 [0.18-0.50]	
Portal Inflammation						
Gender/menopause categories		<0.01		1.00		0.90
Pre-menopause	-				-	
Post-menopause	2.35 [1.63-3.40]		1.00 [0.62-1.61]		0.91 [0.57-1.48]	
Men	1.39 [0.97-2.01]		1.02 [0.67-1.54]		0.98 [0.65-1.49]	

Model 1 includes age, HOMA-IR, waist/BMI, and steatosis as covariates; Model 2 additionally includes white race, hypertension, alcohol consumption, and current smoking.