Published in final edited form as:

Hepatology. 2016 July ; 64(1): 85–91. doi:10.1002/hep.28514.

A longer duration of estrogen deficiency increases fibrosis risk among postmenopausal women with nonalcoholic fatty liver disease

Jagpal Singh Klair[1], **Ju Dong Yang**[2], **Manal F. Abdelmalek**[3], **Cynthia D. Guy**[4], **Ryan M. Gill**[5], **Katherine Yates**[6], **Aynur Unalp-Arida**[7], **Joel E. Lavine**[8], **Jeanne M. Clark**[9], **Anna Mae Diehl**[3], **Ayako Suzuki**[3,10,11], and **for NASH CRN**

[1]Department of Medicine, University of Arkansas for Medical Sciences, Little Rock, AR

[2]Division of Gastroenterology and Hepatology, Mayo Clinic College of Medicine, Rochester, MN

[3]Gastroenterology and Hepatology, Duke University, Durham, NC

[4]Dept. of Pathology, Duke University, Durham, NC

[5]Dept. of Pathology, University of California San Francisco, San Francisco, CA

[6]NASH CRN Data Coordinating Center, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

[7]Division of Digestive Diseases and Nutrition, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD

[8]Dept. of Pediatrics, Columbia University, New York, NY

[9]Division of General Internal Medicine, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

[10]Gastroenterology, Central Arkansas Veterans Healthcare System, Little Rock, AR

[11]Gastroenterology and Hepatology, University of Arkansas for Medical Sciences, Little Rock, AR

Abstract

Post-menopausal women with nonalcoholic steatohepatitis (NASH) are at an increased risk of hepatic fibrosis when compared with premenopausal women. Whether duration of estrogen deficiency in postmenopausal state dictates individual's fibrosis risk remains uninvestigated. We aimed to assess the associations of age at menopause and time from menopause with fibrosis severity in postmenopausal women with nonalcoholic fatty liver disease (NAFLD). Data from 488 post-menopausal women with 1) histologic diagnosis of NAFLD and 2) self-reported information on age at menopause were analyzed. The associations of premature menopause (age at menopause of <40 years) and time from menopause (age at study enrollment - age at menopause, years) with fibrosis severity (stage 0–4) were assessed using multiple ordinal logistic regression models with and without adjusting for clinical confounders. Among the participants (age at menopause: 43.7

Corresponding Author: *Ayako Suzuki, MD, PhD, Msc*, Division of Gastroenterology, Central Arkansas Veterans Healthcare System, 111/HLR, Little Rock, AR 72205, TEL: 501-257-5913; FAX: 501-257-5794, ayako.suzuki@va.gov.

± 8.6 years), women with premature menopause (29.3 %) were younger at enrollment (p<0.001) and used hormone replacement therapy (HRT) more often (p<0.003). After adjusting for age at enrollment, race, waist circumference standardized by body mass index, current smoking, current alcohol use, hypertension, diabetes/impaired fasting glucose, homeostatic model assessment of insulin resistance, and HRT, premature menopause was associated with an increased likelihood of having more severe fibrosis; adjusted cumulative odds ratio and 95% confidence interval (ACOR [95%CI]) was 1.9 [1.3–2.7], p=0.001, while time from menopause was directly associated with an increased likelihood of having more severe fibrosis (ACOR [95%CI] for 5-year unit=1.2 [1.1–1.3], p=0.002).

Conclusion—Duration of estrogen deficiency in postmenopausal state confers fibrosis risk among post-menopausal women with NAFLD.

Keywords

nonalcoholic fatty liver disease; menopause; premature menopause; hepatic fibrosis; nonalcoholic steatohepatitis

INTRODUCTION

Postmenopausal women are at an increased risk of NAFLD. Previous epidemiological studies consistently reported that NAFLD risk increases after age of menopause.(1) This increased risk is likely explained by the loss of estrogen's protective effects after menopause; among post-menopausal women, women under hormone replacement therapy (HRT) were associated with a reduced risk of NAFLD defined by non-viral, non-alcoholic elevation of liver aminotransferases when compared with women without HRT.(2) Postmenopausal women are also at an increased risk of NASH fibrosis. We recently reported that postmenopausal women are at an increased risk of hepatic fibrosis when compared to premenopausal women among patients with NASH.(3) This observation has been independently validated in non-obese, Japanese post-menopausal women.(4) The increased fibrosis risk in postmenopausal women was observed with the adjustment for degrees of hepatocyte ballooning and portal inflammation, two strong histologic predictors for hepatic fibrosis, suggesting that the risk is likely attributed to fibrogenesis, but not hepatocyte ballooning or portal inflammation. Estrogens are known to inhibit stellate cell activation and fibrogenesis in experimental models, which may mechanistically support the clinical observations.(5)

Age at menopause significantly varies among women, depending on race/ethnicity, lifestyles, and genetic factors.(6) Most women experience their menopause between 45 years and 55 years.(6) However, some women experience premature menopause (age <40 years), either due to surgical procedures, chemotherapy, or other unknown causes.(7) Theoretically, a longer duration of estrogen deficiency may dictate a higher fibrosis risk among postmenopausal women with NAFLD. However, whether the duration of estrogen deficiency correlates with fibrosis severity among postmenopausal women with NAFLD remains unknown. In this study we sought to investigate the associations of premature menopause and time from menopause with fibrosis severity among postmenopausal women with

NAFLD with the adjustment for different sets of relevant clinical factors to interpret the associations.

MATERIALS AND METHODS

Study design and Population

This is a cross-sectional study designed to investigate the associations of premature menopause and time from menopause with the severity of fibrosis in post-menopausal women with NAFLD. Data from 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 Nov 2013) were utilized for our analysis.(8–10)

First, postmenopausal women with a centrally reviewed liver biopsy within 6 months of enrollment in the above NASH CRN studies were identified (N=498). The definition of the post-menopausal state was based on self-reported menopause (more than 12 months amenorrhea) via the standardized NASH CRN questionnaire. (8–10) Per the NASH CRN study protocols, patients with serologic or histologic evidence of other forms of chronic liver diseases (viral hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, autoimmune hepatitis, hemochromatosis, Wilson`s disease, or alpha-1 antitrypsin deficiency, hepatocellular carcinoma, or positive for human immunodeficiency virus) were excluded from the studies.(8–10) In the situation in which a participant had more than one study enrollment (i.e., follow-up enrollment), data from the first enrollment (baseline) were used for our analysis. Cases with reported alcohol intake of ≥7 servings per week or those that did not meet histological criteria for NAFLD at the NASH CRN central pathology review were excluded from this analysis (N=3). Seven subjects who did not have the information on age at menopause were also excluded from this analysis, resulting in total of 488 subjects. The NASH CRN studies were approved by the Institutional Review Boards at all sites.

Liver histology

All liver biopsy specimens were stained with hematoxylin-eosin and Masson trichrome and reviewed and scored by the NASH CRN Pathology Committee in a consensus manner according to the NASH CRN scoring system.(11)

Study variables

The primary predictors of this study were premature menopause and time from menopause. Premature menopause was defined as self-reported age at menopause < 40 years old. Time from menopause was calculated as age at study enrollment minus the self-reported age at menopause (years). Surgical menopause was also considered as menopause. The primary outcome was fibrosis stage (stage 0 to 4). For the analysis, the fibrosis stage 1a, 1b, and 1c were combined and treated as a single stage. Other variables taken into account in the analysis include age at enrollment (years), race (White vs. others), waist circumference standardized by body mass index (BMI), current smoking (yes vs. no), current alcohol use (yes vs. no), diagnosis of hypertension, diagnosis of diabetes/impaired fasting glucose, the homeostatic model assessment of insulin resistance (HOMA-IR), current use of hormone

replacement therapy (HRT), and histologic grades of steatosis, lobular inflammation, portal inflammation, hepatocyte ballooning, and Mallory-Denk bodies. HOMA-IR was calculated from fasting insulin and glucose levels using the following formula: [fasting insulin (μU/L)] * [fasting glucose (mg/dL)]/405.(12) All the clinical information was collected at the time of study enrollment using the standardized questionnaires.(8¯10)

Statistical analyses

Data were reported as mean ± standard deviation or median and interquartile range for continuous variables and as a percentage for categorical variables. Clinical and histologic characteristics of the study population were compared in premature menopause vs. others using student t-tests or Wilcoxon rank-sum tests for continuous variables and chi-square tests for categorical variables. Histologic scores, except for Mallory-Denk bodies (Chi-square test), were analyzed using Wilcoxon rank-sum tests to compare the severity of histologic features between the groups.

To assess the associations between the primary study predictors (premature menopause and time from menopause) and severity of NASH fibrosis, a multiple ordinal logistic regression model (MOLR) was developed. Age at enrollment, race, waist circumference standardized by BMI, current smoking, current alcohol use, hypertension, diabetes/impaired fasting glucose, HOMA-IR, and HRT were considered in the models as potential confounders. We also developed a model including grades of hepatocyte ballooning and portal inflammation in addition to the above model. Significance of the associations was determined using likelihood ratio tests. Magnitude of association was expressed as unadjusted or adjusted cumulative odds ratio (COR or ACOR) and 95% confidence interval (CI). As a secondary analysis, we also developed the above-mentioned models including only subjects who had histologic diagnosis of borderline or definite NASH (N=396).

Statistical analyses were performed using JMP statistical software v.9.0 (SAS Institute, Cary, NC) and differences were considered statistically significant when the $P<0.05$. All *P* values presented are 2-sided.

RESULTS

Patient characteristics

Clinical characteristics of the 488 study participants are summarized in Table 1. Mean age ± SD at menopause was 43.7 ± 8.6 yrs. 29.3% women had premature menopause. Women with premature menopause were younger at enrollment (55 ± 8.6 vs. 58.6 ± 6.6 years, $p<0.0001$, t-test) and used HRT more often (26.6% vs. 13.9%, $p=0.001$, Chi-square test).

Fibrosis stage was significantly different between premature menopause vs. others (Table 2). The other histologic variables were not statistically different while grades of hepatocyte ballooning and portal inflammation tended to be higher in women with premature menopause vs. others (Table 2).

Premature menopause and severity of fibrosis

Unadjusted and adjusted associations of premature menopause with hepatic fibrosis stage are shown in Table 3. Premature menopause was associated with 50% increased risk of more severe fibrosis when compared to others (1.5 [1.0–2.1], p=0.03). After adjusting for age at enrollment, race, waist circumference standardized by BMI, current smoking, current alcohol use, hypertension, diabetes/impaired fasting glucose, HOMA-IR, and HRT (Model 1), premature menopause was associated with 90% increased risk of having more severe fibrosis (1.9 [1.3–2.7], p=0.001). Next, we added grades of hepatocyte ballooning and portal inflammation to Model 1 (Model 2) in order to assess the alteration of the association after adjusting for the levels of hepatocyte ballooning and portal inflammation.(3) The ACOR in Model 2 was 1.5 [1–2.3], p=0.03), indicating 44.4% reduction (from 90% to 50%) in the effect size.

The above analysis was repeated after restricting the population to the subjects who had histologic diagnosis of borderline or definite NASH. As shown in Table 4, premature menopause was associated with 60% increased risk of having more severe fibrosis (1.6 [1.1–2.4], p=0.02). After adjusting for the levels of hepatocyte ballooning and portal inflammation, the effect size was reduced 30%.

Time from menopause and severity of fibrosis

Unadjusted and adjusted associations of time from menopause with hepatic fibrosis stage are shown in Table 3. In univariate analysis, time from menopause was associated with an increased risk of more severe fibrosis (COR [95%CI] for 5-year unit = (1.2 [1.1–1.3], <0.0001). After adjusting for age at enrollment, race, waist circumference standardized by BMI, current smoking, current alcohol use, hypertension, diabetes/impaired fasting glucose, HOMA-IR, and HRT (Model 1), time from menopause was still associated with similar increased risk of having more severe fibrosis (COR[95%CI] for 5-year unit = (1.2 [1.1–1.3], p=0.002). After adding degrees of hepatocyte ballooning and portal inflammation to the model 1 (Model 2), time from menopause remained associated with an increased risk (COR [95%CI] for 5-year unit = (1.1 [1.0–1.2], p=0.06), with 50% reduction in the effect size (10% reduction from 20%).

After restricting the population to the subjects who had histologic diagnosis of borderline or definite NASH, the association between time from menopause and hepatic fibrosis stage remained the same (Table 4).

DISCUSSION

In this study we showed that women who had menopause at an earlier age (age at menopause of <40 years, or premature menopause) were at an increased likelihood of having more severe fibrosis when compared with women who had menopause at a later age (age at menopause of ≥ 40 years); women with premature menopause were associated with a 90% increased risk of more severe fibrosis after adjusting for age at enrollment, White race, waist circumference standardized by BMI, current smoking, current alcohol use, hypertension, diabetes/impaired fasting glucose, HOMA-IR, and HRT use. Interestingly, when adding

grades of hepatocyte ballooning and portal inflammation to the model, the effect size was minimally reduced (44.4%). The time from menopause (years) was also directly associated with an increased likelihood of having more severe fibrosis. After restricting the population to subjects with NASH diagnosis, the effect size of premature menopause was reduced by 30%.

Our findings have clinical relevance. Fibrosis risk among patients with NAFLD determines not only the liver outcomes (e.g., cirrhosis and HCC) but also overall outcomes.(13) Thus, risk stratification based on an individual's fibrosis risk is a key to personalizing our care in NAFLD and allocating limited medical resources to the high-risk population. Our analysis suggests that women who experienced their menopause in their earlier age (age of <40 years) are at an even higher risk of hepatic fibrosis and should be followed closely with more aggressive risk modification.

The above-mentioned findings, together with previous reports, underscore the significance of reproductive information for risk stratification among women with NAFLD.(3, 4) Not only menopausal status, but also age at menopause and time from menopause appear to determine fibrosis risk among women with NAFLD. Our study suggests that the longer time of estrogen depletion, the higher risk of having more severe fibrosis among postmenopausal women. Postmenopausal women are known to be at an increased risk of NAFLD and metabolic features of insulin resistance; increased total as well as visceral adiposity in peri- and post-menopause are associated with an increased risk of insulin resistance, dyslipidemia, hypertension, diabetes, and cardiovascular disease.(1) Further, given protective effects of estrogens against oxidative stress and fibrogenesis, postmenopausal women may be at an increased risk of portal inflammation, ballooning and fibrosis at a given metabolic stress in a setting of NAFLD.(1) The observed association in this study remained significant (even stronger) after adjusting for relevant clinical confounders such as visceral adiposity, metabolic features, and insulin resistance. Our secondary analysis in which we restricted the population to patients with NASH showed 30% risk reduction (1.9 to 1.6 as ACOR). This may imply that a third of the risk among postmenopausal women might be attributed to the risk of having NASH. Further, when we added grades of hepatocyte ballooning and portal inflammation to the model, the effect size was reduced by 44.4%. Further, when we ran a logistic regression model with significant fibrosis (stage 2–4) as an outcome, the results were basically same (data not shown). Premature menopause was associated with 90% increased risk of having significant hepatic fibrosis vs. others after adjusting for the same variable set and the effect size was decreased by only 20% after adjusting for hepatocyte ballooning and portal inflammation. These data suggest that the majority of the 'effect' may be explained by fibrogenesis (or fibrosis regression), rather than insulin resistance or activity of ballooning or portal inflammation. Future prospective follow-up study is warranted to test the above hypothesis.

Given the estrogen's protective effect against fibrogenesis(5) and the clinical observations(3, 4), one may anticipate protective effect of HRT on fibrogenesis. Our prior analysis showed a borderline beneficial effect of estrogen replacement therapy on fibrosis (50%).(3) In this study, our analysis did not show any beneficial effect of estrogen replacement therapy on fibrosis in any model or subgroup (premature menopause or others). Despite the theoretical

beneficial effect of estrogens on fibrogenesis, whether estrogen replacement therapy with synthetic estrogens reduces fibrosis risk among postmenopausal women remains uncertain and inconclusive. Further analyses incorporating the information on age at initiation, dose, route of administration, and duration of estrogen replacement therapy would be helpful to better characterize potential beneficial effect of estrogens on fibrosis among post-menopausal women with NAFLD and also to determine whether or not the beneficial effect of estrogens is limited to a specific subgroup of post-menopausal women.

This study has several limitations. Cross-sectional study design precludes us from addressing causality. Our study population was enrolled at tertiary academic centers. Thus, there might be potential selection bias and the findings should be interpreted with caution. Clinical and diagnostic information to discern whether or not patients with premature menopause met criteria for co-existing diagnosis of polycystic ovary syndrome (14) or obstructive sleep apnea are lacking. Whether the presence of polycystic ovary syndrome in patients with NAFLD and/or hyperandrogenemia contributes to our finding will require further investigation. Lastly, detailed information on HRT, such as age at initiation, dose, route of administration, and duration, was not available in this study. Thus, as discussed above, potential impact of HRT on fibrosis among postmenopausal women is still inconclusive, pending future investigation. Further extending our research question, one may ask numbers of pregnancies, duration of breast feeding, or duration of oral contraceptive use may influence fibrosis risk among women. These questions could not be addressed in our analysis, pending future investigation.

In summary, our analysis revealed that not only menopausal status, but also age at menopause, and duration of estrogen depletion (time from menopause) appear to be significant fibrosis risk determinants among women with NAFLD. Our findings further emphasize the importance of reproductive history in risk stratification among female patients with NAFLD and in personalized medicine. Whether HRT is beneficial for the prevention of fibrosis progression among postmenopausal women remains uncertain. Further investigation is warranted to establish an effective prevention for this high-risk population.

Acknowledgments

Disclosures and Conflicts of Interest:

The NASH CRN is supported by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) (grants U01DK061718, U01DK061728, U01DK061731, U01DK061732, U01DK061734, U01DK061737, U01DK061738, U01DK061730, U01DK061713).

In addition, support from the National Center for Advancing Translational Sciences (NCATS) in conduct of NASH CRN Studies (grants UL1TR000439, UL1TR000077, UL1TR000436, UL1TR000150, UL1TR000424, UL1TR000006, UL1TR000448, UL1TR000040, UL1TR000100, UL1TR000004, UL1TR000423, UL1TR000058, UL1TR000067, UL1TR000454).

Abbreviations

NAFLD	nonalcoholic fatty liver disease
NASH	nonalcoholic steatohepatitis

NASH CRN	Nonalcoholic Steatohepatitis Clinical Research Network (CRN)
HRT	hormone replacement therapy
COR	Cumulative odds ratio
CI	confidence interval
HOMA-IR	homeostatic model assessment of insulin resistance
DM	Diabetes mellitus
HCC	Hepatocellular carcinoma
BMI	body mass index
DB	Database
MOLR	multiple ordinal logistic regression model
ACOR	adjusted cumulative odds ratio
HTN	Hypertension

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Table 1

Clinical Characteristics of the Study population

	Premature Menopause (N = 143)	Others (N = 345)	P value**
Age of enrollment, years	55 ± 8.6	58.6 ± 6.6	< 0.001
Age at menopause, years	32.5 ± 4.7	48.3 ± 4.8	<0.001
Race, %			0.98
White	111 (77.6%)	265 (77.3%)	
Others	32 (22.4%)	80 (22.7%)	
Ethnicity – Hispanics, %	14 (9.7%)	29 (8.4%)	0.63
BMI, kg/m^2	34.5 ± 6.2	34.3 ± 6.8	0.72
Waist, cm	107.6 ± 13.8	106.6 ± 13.9	0.44
Diabetes/Impaired glucose tolerance, %	78 (54.6%)	208 (60.3%)	0.24
HOMA-IR	7.1± 6.4	7.0 ± 7.7	0.95
Hypertension, %	118 (82.5%)	274 (79.4%)	0.43
Current smokers, %	17 (11.9%)	28 (8.1%)	0.20
Alcohol consumption*, %	55 (38.5%)	141 (40.9%)	0.62
Treatment with HRT, %	38 (26.6%)	48 (13.9%)	0.001

*Alcohol intake was defined as any reported alcohol consumption but less than 7 drinks per week (alcohol consumption of 7 or more drinks per week was exclusionary for the study enrollment and retention)..

**p-values from Chi-square test, Student's t-test or Wilcoxon rank sum test

Table 2

Histologic Features of the Study population

	Premature Menopause (N = 143)	Others (N = 345)	P value
Steatosis			0.34
Grade 0	7 (5%)	14 (4%)	
Grade 1	63 (44%)	140 (40.6%)	
Grade 2	43 (30%)	108 (31.4%)	
Grade 3	30 (21%)	83 (24%)	
Lobular inflammation			0.26
Grade 0	0 (0%)	1 (0.3%)	
Grade 1	74 (51.7%)	194 (56.2%)	
Grade 2	50 (35%)	115 (33.3%)	
Grade 3	19 (13.3%)	35 (10.2%)	
Hepatocyte ballooning			0.06
Grade 0	33 (23%)	106 (30.7%)	
Grade 1	35 (24.5%)	87 (25.2%)	
Grade 2	75 (52.5%)	152 (44.1%)	
Mallory-Denkbody			0.17*
No	82 (57.3%)	221 (64%)	
Yes	61 (42.7%)	124 (36%)	
Portal inflammation			0.10
Grade 0	10 (7%)	36 (10.4%)	
Grade 1	82 (57.3%)	210 (60.9%)	
Grade 2	50 (35%)	99 (28.7%)	
Fibrosis			0.03
Stage 0	22 (15.4%)	84 (24.4%)	
Stage 1	34 (23.8%)	84 (24.4%)	
Stage 2	32 (22.4%)	63 (18.3%)	
Stage 3	35 (24.4%)	78 (22.6%)	
Stage 4	20 (14%)	35 (10.1%)	

P-values are from Wilcoxon rank sum tests except for * (Chi-square test).

Table 3

Premature menopause, Time from menopause and risk of Fibrosis

	Univariate COR(95%CI), p-value	**Model 1 COR(95%CI), p-value**	**Model 2 COR(95%CI), p-value**
Premature menopause			
No	-	-	-
Yes	1.5 [1.0–2.1], p=0.03	1.9 [1.3–2.7], p=0.001	1.5 [1.0–2.3], p=0.03
Time from menopause, for 5 years	1.2 [1.1–1.3], p<0.0001	1.2 [1.1–1.3], p=0.002	1.1 [1.0–1.2], p=0.06

Table 4

Premature menopause, Time from menopause and risk of Fibrosis: subgroup analysis in patients with borderline NASH or NASH

	Univariate COR(95%CI), p-value	**Model 1 COR(95%CI), p-value**	**Model 2 COR(95%CI), p-value**
Premature menopause			
No	-	-	-
Yes	1.2 [0.9–1.8], p=0.27	1.6 [1.1–2.4], p=0.02	1.4 [0.9–2.1], p=0.11
Time from menopause, for 5 years	1.2 [1.1–1.4], p<0.0001	1.2 [1.0–1.3], p=0.01	1.1 [1.0–1.3], p=0.05

Model 1: Adjusted for age at enrollment, race, waist circumference/BMI, current smoking/alcohol use, hypertension, diabetes/IFG, HOMA-IR, and HRT.

Model 2: Adjusted for the above covariates, hepatocyte ballooning grades, and portal inflammation grades.