

High Hemoglobin Level Predicts Non-alcoholic Fatty Liver Disease in Japanese Women

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Received: April 2, 2013 Revised: May 1, 2013

Accepted: May 3, 2013

Published online: June 21, 2013

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Key words: NAFLD; Hemoglobin; Menopause; Ferritin; Insulin resistance

Akahane T, Fukui K, Shirai Y, Yoshiji H, Uemura M, Hiroshi Fukui. High Hemoglobin Level Predicts Non-alcoholic Fatty Liver Disease in Japanese Women. *Journal of Gastroenterology and Hepatology Research* 2013; 2(6): 623-627 Available from: URL: <http://www.ghrnet.org/index.php/joghr/article/view/401>

INTRODUCTION

Obesity-related liver disease, particularly non-alcoholic fatty liver disease (NAFLD), is increasingly common in developed countries, and represents the hepatic manifestation of metabolic syndrome. NAFLD includes a spectrum of liver tissue alterations ranging from simple hepatic steatosis to hepatitis and progressive fibrosis, and is associated with a high risk of type 2 diabetes and cardiovascular disease^[1,2].

In Japan, the prevalence of NAFLD is higher in men than women in the population aged <50 years. The prevalence of NAFLD increases rapidly in older women to equal or surpass that in men^[3]. This suggests that menopause is associated with an increase in the risk of NAFLD, and that ovarian hormones help to protect premenopausal women. The prevalence of obesity increases after menopause. The low estrogen level in postmenopausal women is associated with loss of subcutaneous fat and gain of visceral fat^[4-6]. Increased visceral fat is an independent predictor of NAFLD, and is much more harmful than subcutaneous fat^[7,8]. Weight gain after menopause therefore increases the risk of NAFLD, and body mass index (BMI) is a useful marker for predicting NAFLD. However, not all obese women develop NAFLD.

The frequent association between hepatic steatosis and body iron overload is known as insulin resistance-associated hepatic iron overload^[9]. Hyperferritinemia associated with mild hepatic iron accumulation is frequently observed in NAFLD^[10-12]. Insulin stimulates ferritin synthesis and facilitates iron uptake^[13], and conversely, iron influences insulin signaling^[14], reduces hepatic extraction and metabolism of insulin which leads to peripheral hyperinsulinemia^[15], and may increase cellular oxidative stress, which inhibits the internalization and actions of insulin^[16].

ABSTRACT

AIM: To identify whether hemoglobin (Hb) level is a predictor of non-alcoholic fatty liver disease (NAFLD) in Japanese women.

METHODS: The subjects were 1673 Japanese women (mean age: 52.1±8.7 years, age range: 30-82 years) who underwent a physical checkup during 2009. Women with hepatitis B or C virus infection, other hepatobiliary disease or alcohol consumption >20 g/day were excluded from the study. NAFLD was diagnosed by ultrasonography. Alanine aminotransferase (ALT) >30 U/L was defined as elevated ALT. Ferritin level was measured in 100 selected subjects to examine the relationship between Hb and ferritin levels.

RESULTS: NAFLD was diagnosed in 245 subjects and NAFLD with elevated ALT were detected in 73 subjects. Hb level was significantly higher in subjects with NAFLD than those without NAFLD. BMI, menopausal status and Hb, triglycerides and glucose levels were independently associated with ALT level. Logistic regression analysis showed that BMI [odds ratios (OR) =1.54, 95% CI 1.45-1.63], menopausal status (OR=1.98, 95% CI 1.34-2.74) and Q4 (top 25th percentile) Hb level (OR=2.18, 95% CI 1.36-3.50) were associated with NAFLD. Q3 (51st-75th percentiles) Hb level (OR=2.71, 95% CI 1.07-6.87) and Q4 Hb level (OR=2.69, 95% CI 1.07-6.76) were associated with NAFLD with elevated ALT. Multivariate analysis showed that Hb level was independently associated with ferritin level and insulin resistance.

CONCLUSION: High Hb level is a predictor of NAFLD. Hb level is associated with iron stores and insulin resistance in Japanese women.

Premenopausal women have lower hepatic iron stores because of iron loss during menstruation and increased requirements during pregnancy. The body iron stores increase after menopause^[17], which may contribute to the increased risk of NAFLD in postmenopausal women.

Liver enzymes are not considered to be sensitive or specific for diagnosing NAFLD, and a significant number of NAFLD patients may have normal enzymes. Although serum ferritin level is a reliable marker for hepatic iron stores, it is not measured as a screening test during physical checkups. If there are useful markers besides BMI which can predict NAFLD, it would be possible to detect patients with NAFLD more accurately. We focused on hemoglobin (Hb) level instead of ferritin level, because Hb level is often measured as a screening test. Hb is a predominant component in erythrocytes and functions as an oxygen transporter in blood. Recently Hb expression was reported in hepatocytes and was increased in NASH^[18]. The purpose of this study was to investigate whether Hb level is a predictor of NAFLD in Japanese women.

METHODS

Our cross-sectional study used data from 1825 women (age range: 30-82 years), who had undergone physical checkups at Nara Health Promotion Center during 2009. Of these, 152 were excluded from the study for the following reasons: hepatitis B surface antigen or anti-hepatitis C virus antibody positive, other hepatobiliary disease or alcohol consumption of >20 g/day. The remaining 1673 participants (934 postmenopausal women and 739 premenopausal women, mean age: 52.1±8.7 years, age range: 30-82 years) were included in this study. One hundred women (mean age: 52.6±7.8 years, age range: 31-74 years) were selected from this group for measurement of ferritin levels. This study was approved by the Ethics Committee of Nara Health Promotion Center. All subjects gave informed consent.

Each subject underwent a complete physical examination including anthropometric measurements. Height and body weight were measured using a digital scale, with the subject wearing a light gown. BMI was calculated as weight (kg)/height squared (m²). Subjects were asked not to ingest any food for 12 h or water for 2 h prior to the examination. Resting blood pressure was measured using an automatic sphygmomanometer. All biochemical analyses were performed within 2 h after obtaining blood samples, in the same laboratory, by standard methods using commercially available kits and an automated analyzer. Alanine aminotransferase (ALT)>30 U/L was defined as elevated ALT. The Hb level was classified into 4 quartiles: Q1 (0th-25th percentiles), Q2 (26th-50th percentiles), Q3 (51st-75th percentiles), Q4 (76th-100th percentiles). Insulin resistance was assessed using the homeostasis model assessment (HOMA) of insulin resistance=fasting insulin (μU/mL)×fasting glucose (mg/dL)/405. NAFLD was defined by ultrasonography when there was increased liver echogenicity ("bright liver") and contrast between the hepatic and renal parenchymal tissues^[19].

Statistical analysis

Data are expressed as the mean±standard deviation. The Student's *t*-test was used to compare quantitative variables with a normal distribution. The Chi-square test was used to compare qualitative variables. Odds ratios and 95% confidence intervals for NAFLD risk factors were calculated using the standard logistic regression model. Multivariate analysis was performed based on the results of univariate analyses. A *p*-value of <0.05 was considered to be statistically significant. All calculations were performed using SPSS software (Version 18.0, Inc., Chicago, IL).

RESULTS

Clinical characteristics

NAFLD was diagnosed in 245 subjects, of which 180 were postmenopausal. The mean age was higher in subjects with NAFLD than those without. Waist circumference, BMI, blood pressure, insulin resistance, and triglyceride (TG), glucose, insulin and CRP levels were significantly higher in subjects with NAFLD than those without. HDL cholesterol was significantly lower in subjects with NAFLD than those without. Hb and hematocrit (Ht) levels were significantly higher in subjects with NAFLD than those without (Table 1 and Figure 1).

Table 1 Anthropometric, clinical and laboratory data of the study population.

	With NAFLD <i>n</i> =245	Without NAFLD <i>n</i> =1428
Age (year)	55.3±7.1 ¹	51.6±8.8
Menopause [<i>n</i> (%)]	180 (73.5%) ¹	754 (52.8%)
Waist circumference (cm)	89.0±8.2 ¹	78.5±7.8
BMI (Kg/m ²)	25.6±3.4 ¹	21.6±2.8
SBP (mmHg)	130.8±15.4 ¹	120±15.7
DBP (mmHg)	80.9±10.0 ¹	73.9±9.9
AST (U/L)	24.5±10.5 ¹	20.4±7.2
ALT (U/L)	28.8±18.7 ¹	17.4±10.2
GGT (U/L)	29.8±18.5 ¹	20.9±23.0
Triglycerides (mg/dL)	123.1±51.6 ¹	78.3±35.5
HDL (mg/dL)	59.7±14.5 ¹	69.1±14.1
Glucose (mg/dL)	102.8±22.5 ¹	90.7±9.7
Insulin (μU/mL)	9.5±8.6 ¹	4.8±2.3
HOMA	2.5±2.1 ¹	1.1±0.97
CRP	0.11±0.11 ²	0.06±0.22
Red blood cells (10 ⁴ /μL)	461±36 ¹	443±32
Hemoglobin (g/dL)	13.4±1.2 ¹	12.9±1.2
Hematocrit (%)	41.4±3.1 ¹	40.0±3.1
Platelet (10 ⁴ /μL)	25.4±5.8 ¹	23.4±5.2
Current smokers [<i>n</i> (%)]	8 (3.3%)	59 (4.1%)

SBP: Systolic blood pressure; DBP: Diastolic blood pressure; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: γ -glutamyltransferase; HDL: High-density lipoprotein-cholesterol; HOMA: Homeostasis model assessment of insulin resistance. ¹ *p*<0.001, ² *p*<0.05 between subjects with NAFLD and those without.

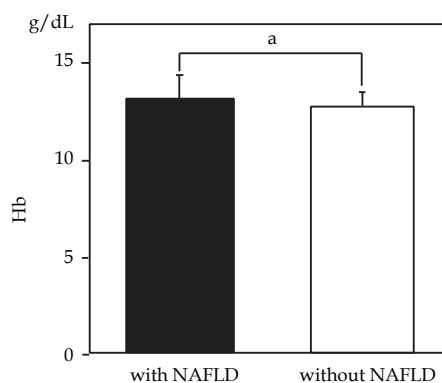


Figure 1 Comparison of hemoglobin (Hb) level between subjects with NAFLD and those without. ^a *p*<0.001 between subjects with NAFLD and those without.

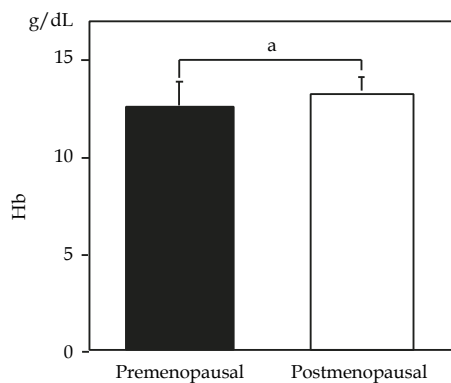
Comparison between pre- and postmenopausal women

The prevalence of NAFLD was significantly higher in postmenopausal than premenopausal women. Risk factors for metabolic syndrome such as increased waist circumference, BMI, blood pressure, insulin resistance, and TG and glucose levels were significantly more frequent in postmenopausal than premenopausal women. AST, ALT, GGT, Hb and Ht levels were also significantly higher in postmenopausal than premenopausal women (Table 2 and Figure 2).

Table 2 Comparisons of anthropometric, clinical and laboratory data between pre- and postmenopausal women.

	Premenopausal women <i>n</i> =739	Postmenopausal women <i>n</i> =934
NAFLD [<i>n</i> (%)]	65 (8.8%) ³	180 (19.3%)
Waist circumference (cm)	78.1±8.2 ³	81.6±8.8
BMI (Kg/m ²)	21.7±2.9 ³	22.5±3.1
SBP (mmHg)	117.1±14.7 ³	124.7±16.4
DBP (mmHg)	72.2±9.5 ³	77.0±10.2
AST (U/L)	18.8±6.0 ³	22.8±8.7
ALT (U/L)	16.4±9.8 ³	21.2±14.0
GGT (U/L)	18.0±17.9 ³	25.6±25.4
Triglycerides (mg/dL)	73.5±36.9 ³	93.4±42.6
Glucose (mg/dL)	90.0±9.5 ³	94.4±15.1
HOMA	1.2±0.8 ⁴	1.4±1.3
Red blood cells (10 ⁴ /μL)	444±33	447±33
Hemoglobin (g/dL)	12.5±1.4 ³	13.3±0.9
Hematocrit (%)	39.1±3.5 ³	41.1±2.5
Platelet (10 ⁴ /μL)	25.0±5.9 ³	22.7±4.6
Current smokers [<i>n</i> (%)]	35 (4.7%)	32 (3.4%)

SBP: Systolic blood pressure; DBP: Diastolic blood pressure; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: γ-glutamyltransferase; HOMA: Homeostasis model assessment of insulin resistance. ³*p* < 0.001, ⁴*p* < 0.05 between pre- and postmenopausal groups.

**Figure 2** Comparison of hemoglobin (Hb) level between pre- and postmenopausal women. ^a*p* < 0.001 between subjects with pre- and postmenopausal women.

Factors correlated with ALT level

We used multivariate analysis to analyze factors correlated with ALT level. High BMI, postmenopausal status, and high TG, glucose and Hb levels were identified as independent predictors of high ALT level (Table 3).

Table 3 Results of multivariate analysis of relationships between alanine aminotransferase level and clinical characteristics.

	β	<i>p</i>
BMI	0.152	< 0.001
SBP	0.028	NS
Triglycerides	0.070	0.006
Glucose	0.109	<0.001
Hb	0.104	<0.001
Menopause	0.097	<0.001

NS: Nonsignificant; SBP: Systolic blood pressure; Hb: Hemoglobin.

Factors associated with NAFLD

Logistic regression analysis with NAFLD as the dependent variable showed that postmenopausal status, high BMI and Q4 Hb level were significantly associated with an increased risk of NAFLD (Table 4). Furthermore, the statistically significant risk factors for NAFLD with elevated ALT were high BMI, postmenopausal status and Q3 and Q4 Hb levels (Table 5).

Table 4 Logistic regression analysis with NAFLD as a dependent variable.

	NAFLD [<i>n</i> (%)]	Odds ratio (95%CI)	<i>p</i>
BMI (kg/m ²)		1.54 (1.45-1.63)	<0.001
Menopausal status			
Pre menopause	65 (8.8%)	1	
Post menopause	180 (19.3%)	1.98 (1.34-2.74)	<0.001
Hemoglobin (g/dL)			
Q1	38 (8.5%)	1	
Q2	37 (9.0%)	0.79 (0.46-1.35)	0.388
Q3	67 (16.2%)	1.34 (0.82-2.19)	0.246
Q4	103 (25.8%)	2.18 (1.36-3.50)	0.001

CI: Confidence interval.

Table 5 Results of Logistic regression analysis with NAFLD with elevated alanine aminotransferase (ALT) as a dependent variable.

	NAFLD with ALT elevation [<i>n</i> (%)]	Odds ratio (95%CI)	<i>p</i>
BMI (kg/m ²)		1.47 (1.36-1.58)	<0.001
Menopausal status			
Pre menopause	15 (2.0%)	1	
Post menopause	58 (6.2%)	2.34 (1.20-4.56)	0.012
Hemoglobin (g/dL)			
Q1	7 (1.6%)	1	
Q2	7 (1.7%)	0.91 (0.30-2.77)	0.861
Q3	26 (6.3%)	2.71 (1.07-6.87)	0.036
Q4	33 (8.3%)	2.69 (1.07-6.76)	0.035

ALT: alanine aminotransferase; CI: Confidence interval.

Relationship between insulin resistance assessed by HOMA and Hb level

We investigated the correlation between Hb level and insulin resistance. Multivariate analysis showed that BMI and Hb level were independent predictors of insulin resistance (Table 6).

Table 6 Results of multivariate analysis of relationships between insulin resistance assessed by HOMA and age, BMI and hemoglobin level.

	β	<i>p</i>
Age	-0.019	0.405
BMI (kg/m ²)	0.421	<0.001
Hemoglobin (g/dL)	0.089	<0.001

HOMA: Homeostasis model assessment of insulin resistance.

Relationship between ferritin and Hb levels in 100 selected subjects

Thirty-eight of the 100 subjects selected for measurement of ferritin level were premenopausal. NAFLD was diagnosed in 52 subjects, of which 37 (71.1%) were postmenopausal. Ferritin level was significantly higher in postmenopausal than premenopausal women (68.7±52.1 versus 28.9±33.6 ng/mL, *p*<0.001). Univariate analysis showed that Hb level correlated with ferritin level, but BMI did not correlate with ferritin level. Multivariate analysis showed that Hb level was an independent predictor of ferritin level (Table 7).

Table 7 Results of univariate and multivariate analysis of relationships between ferritin level and age, BMI and Hb level.

	Univariate analysis		Multivariate analysis	
	β	<i>p</i>	β	<i>p</i>
Age	0.366	<0.001	0.316	0.001
BMI (kg/m ²)	0.117	0.250	0.015	0.873
Hb (g/dL)	0.399	<0.001	0.352	<0.001

Hb: Hemoglobin.

DISCUSSION

The major finding of this study is that a high Hb level is an independent predictor of NAFLD in Japanese women. We found that Hb level was higher in subjects with NAFLD than those without. Multivariate analysis showed that Hb level correlated with both

ferritin level and insulin resistance assessed by HOMA, indicating a high probability that Hb level is associated with iron stores and insulin resistance.

We analyzed Hb levels of 1914 men, who had undergone physical checkups at our center during 2009 (Individuals with hepatitis B surface antigen or anti-hepatitis C virus antibody positive, other hepatobiliary disease or alcohol consumption of >20 g/day were excluded). Univariate analysis showed that Hb level was negatively associated with age ($\beta=-0.19$, $p<0.001$). Age did not differ significantly between subjects with NAFLD ($n=719$) and those without, while Hb level was significantly higher in subjects with NAFLD than those without (15.4 ± 0.9 versus 14.8 ± 1.0 g/dL, $p<0.001$) (unpublished data). Therefore, age did not correlate with NAFLD or Hb level in Japanese men and high Hb level may be predictive of NAFLD in both Japanese women and men.

Patients with NAFLD often have a high serum ferritin level. Zelber-Sagi *et al.*^[20] demonstrated that NAFLD is a major determinant of increased serum ferritin level. They also showed that the association between serum ferritin and insulin levels is much more evident in patients with NAFLD than those without. Recent studies have suggested that serum ferritin level is a marker for insulin resistance^[21-23]. Although serum ferritin level is a useful marker for NAFLD, it is not measured in screening tests at the time of physical checkups. However, Hb level is often measured in screening tests, and our results showed that Hb level was independently correlated with ferritin level in 100 subjects, and with insulin resistance assessed by HOMA in all subjects. Our results suggest that Hb level can be as useful a marker for NAFLD as ferritin level.

A recent proteomic study has shown that free Hb α and β subunits in serum were significantly increased from normal controls to steatosis and to NASH, suggesting free Hb subunits in serum could be a biomarker for liver lesions^[24]. In another population study, higher free serum Hb has been associated with higher prevalence rates of NAFLD^[25]. The source of free Hb in serum was not identified, but presumed to result from oxidative stress-induced hemolysis. On the other hand, Liu *et al.*^[18] reported that Hb was expressed in hepatocytes and was increased in NASH. They suggested that elevated oxidative stress in NASH could induce Hb expression and suppression of oxidative stress by Hb could be a mechanism to protect hepatocytes from oxidative damage.

Obstructive sleep apnea is a possible comorbidity in patients with morbid obesity, and is also associated with a high Hb level^[26]. Sleep apnea is rare in Japanese women, and none of the subjects in this study suffered from sleep apnea. Sleep apnea is therefore not a confounder in this study. Smoking might also be associated with an increased Hb level. A national survey in 2009 reported that the smoking rate in Japanese women was 10.9%^[27]. Univariate analysis in this study did not show an association between smoking and Hb level (data not shown), and there were no significant differences in the proportions of smokers between subjects with NAFLD and those without, or between pre- and postmenopausal women (Tables 1 and 2). The likelihood of increased Hb levels in this study having been caused by smoking is therefore probably small.

In conclusion, high Hb level is an independent predictor of NAFLD in Japanese women. Our findings suggests that Hb level can be a useful marker for NAFLD.

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