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Original article

Serum folic acid levels are associated with the presence and severity of liver steatosis in Chinese adults

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SUMMARY

Background & aims: Non-alcoholic fatty liver disease (NAFLD) is a common and strong risk factor for cardiovascular disease and hepatocellular carcinoma. The rapid acceleration of the increase in NAFLD prevalence has exceeded the trends observed for obesity, and has been driven by multiple factors. The aim of this study was to investigate the correlation between the serum levels of folic acid, the endogenous source of methyl groups for DNA methylation, and NAFLD in Chinese adults.

Methods: The correlations between the serum folic acid levels and NAFLD were investigated in two independent cohorts of 70 subjects who underwent a liver biopsy and 130 subjects with varying liver fat contents, as measured using proton magnetic resonance spectroscopy (^{1}H-MRS). Independent correlations between serum folic acid levels and liver steatosis grades were detected using a multivariate ordinal regression analysis. The diagnostic performances of serum folic acid levels alone and in combination with existing NAFLD prediction scores were compared with those of traditional NAFLD prediction parameters using receiver operating characteristic (ROC) curve analyses.

Results: Serum folic acid concentrations were inversely correlated with liver histological steatosis grades ($\rho = -0.371$, $P < 0.001$) and the ^{1}H-MRS-measured liver fat content ($r = -0.199$, $P = 0.038$). According to the multivariate ordinal regression analysis, serum folic acid levels were inversely correlated with liver steatosis grades (OR 0.739 [0.594–0.918], $P = 0.006$) independent of age, gender, BMI, components of metabolic syndrome and the serum TC, LDL-c and HOMA-IR levels. The AUROC of serum folic acid for the diagnosis of NAFLD was 0.75 (0.65–0.83), and the addition of serum folic acid to NAFLD prediction scores significantly improved the diagnostic prediction of NAFLD (AUROC = 0.88 [0.81–0.94]).

Conclusion: Low serum folic acid levels were identified as an independent risk factor for NAFLD in the Chinese population. The addition of the serum folic acid levels to the current existing NAFLD prediction scores significantly improved the prediction of NAFLD.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is currently the most common cause of chronic liver disease worldwide, with an estimated prevalence of 20–30% [1]. As the hepatic manifestation of metabolic syndrome [2], NAFLD increases the risks of cardiovascular disease and type 2 diabetes [3,4] and can progress to advanced liver disease, cirrhosis and hepatocellular carcinoma if it is accompanied by different degrees of liver inflammation and fibrosis [5,6]. Strikingly, the prevalence of NAFLD is still currently increasing, particularly in adolescents [7], despite the relative stability of the obesity epidemic from 2003–2004 to 2013–2014 [8,9]. The accelerated growth of the NAFLD prevalence over time has been driven by obesity and multiple factors related to NAFLD [10]. Based on emerging evidence, epigenetic mechanisms influence the development of NAFLD [11], and DNA methylation is the most intensively studied epigenetic mechanism that modulates susceptibility to NAFLD [12]. Methylome studies have observed pronounced global DNA hypomethylation and aberrant DNA

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Abbreviations

NAFLD	non-alcoholic fatty liver disease
^{1}H-MRS	proton magnetic resonance spectroscopy
NAS	non-alcoholic fatty liver disease activity score
BMI	body mass index
TC	total cholesterol
HDL	high-density lipoprotein
TG	triglycerides
ALT	alanine amino transaminase
AST	aspartate amino transaminase
LDL	low-density lipoprotein
HOMA-IR	homeostatic model assessment of insulin resistance
ROC	receiver operating characteristic
AdoMet	S-adenosylmethionine
AdoHcy	S-adenosylmethionine
PC	phophatidylcholine
VLDL	very low-density lipoprotein
AMPK	5′ AMP-activated protein kinase

methylation at specific gene promoter regions in subjects with steatosis and NASH [13]. Therefore, the biological methylation function in the liver is important for the maintenance of hepatic lipid metabolism [14].

Folic acid, also called vitamin B9, is the endogenous source of methyl groups for hepatic DNA methylation, and may play an important role in the liver lipid metabolism [15]. Animal studies have shown that folate deficiency affects hepatic lipid storage and metabolism resulting in the development of NAFLD [16,17] and liver injury [18], and dietary supplementation with folic acid increases the DNA methylation status and reduces liver fat content in a mouse model [19,20]. In humans, the reported associations between serum folic acid levels and NAFLD are inconsistent and appear to depend on the potential influences of confounding factors, such as gender, age, ethnicity, and body weight [21–24].

As the largest ethnic group in the world, the Han Chinese are more prone to fat accumulation in the liver [25]. Despite an unparalleled 50% increase in the overall prevalence of obesity in China over the past decade [26], the prevalence of NAFLD doubled during that same period [27]. As the serum folic acid levels are deficient in approximately 20% of the Chinese adults [28], the serum folic acid level and the mechanism of DNA methylation should play an indispensable role in the pathogenesis of NAFLD in Chinese adults. However, the association between serum folic acid levels and the severity of NAFLD in the Chinese population has not previously been investigated.

In the present study, we (1) analysed the relationship between serum folic acid levels and the severity of hepatic steatosis in 70 hospitalized subjects who were suspected to have NAFLD and 130 diabetic patients with varying liver fat content, as measured using proton magnetic resonance spectroscopy (^{1}H-MRS), and (2) assessed the diagnostic performance of serum folic acid levels in predicting NAFLD and its clinical use in combination with other known NAFLD risk factors.

2. Materials and methods

2.1. Subjects

We conducted this cross-sectional study with two cohorts from the Inpatient Department of Endocrinology and Metabolism, Zhongshan Hospital (Shanghai, China). Seventy-one patients (34 men and 37 women) who initially enrolled in the study underwent a liver biopsy examination. All patients were diagnosed with fatty liver by ultrasonography, were clinically considered to have NAFLD, and had risks for steatohepatitis. Liver biopsies were performed in patients with metabolic syndrome (n = 63) or chronic elevation of transaminase levels (n = 8) [29]. After excluding one patient with pathological autoimmune hepatitis, 70 patients (33 men and 37 women) were included in the analysis. Another group of 130 eligible Chinese diabetic subjects with varying degrees of NAFLD, as determined by ^{1}H-MRS were also enrolled. The patients included in the study were selected based on the following criteria: (1) no known acute or chronic disease with the exception of obesity or T2DM, based on a medical history, physical examination and laboratory tests conducted during hospitalization (including blood cell counts, serum creatinine, thyroid hormone, and concentrations of electrolytes, hepatitis B and C antibody and autoimmune hepatitis antibody levels); (2) no excessive alcohol consumption (≥140 g per week for men and ≥70 g per week for women) [30]; and (3) no use of hepatic protectants, hepatotoxic agents, folic acid or vitamin B12 supplements in recent years. The clinical characteristics of the two groups of participants are shown in Table 1. The study was approved by the Research Ethics Committee of Zhongshan Hospital, Fudan University, and all participants provided informed written consent prior to participating in the study.

2.2. Histological examination of the liver tissue

A liver biopsy was performed by trained operators using an ultrasound-guided 1.6-mm-diameter needle. All liver tissue samples were examined by an experienced pathologist (Hu XQ) who was blinded to the study design. The NAS score (non-alcoholic fatty liver disease activity score) was used to assess hepatic histology based on a previously described standardized grading system [31], which included steatosis (scale of 0–3), lobular inflammation (scale of 0–3), hepatocellular ballooning (scale of 0–2) and fibrosis (scale of 0–4). Steatosis was graded as S0 (0–5%), S1 (6–33%), S2 (34–66%), or S3 (67–100%).

Table 1
Baseline characteristics of the study population.

	Diagnostic method for NAFLD	
	Liver biopsy	^{1}H-MRS
Number	70	130
Age, year	46.4 ± 14.6	51.6 ± 14.8
Gender (M/F)	33/37	69/61
BMI, kg/m^2	28.4 ± 5.0	26.7 ± 4.4
Waist, cm	95.3 ± 11.9	94.0 ± 10.5
SBP, mmHg	128.5 ± 13.5	131.3 ± 16.7
DBP, mmHg	79.7 ± 9.2	80.8 ± 9.5
FBG, mmol/L	6.1 ± 1.8	7.0 ± 2.5
TG, mmol/L	1.8(1.3–2.4)	1.8(1.2–2.4)
TC, mmol/L	4.6 ± 1.1	4.5 ± 1.1
HDL-c, mmol/L	1.1 ± 0.3	1.0 ± 0.2
LDL-c, mmol/L	2.6(2.0–3.1)	2.6(2.0–3.1)
ALT, U/L	70(38–96)	36(16–77)
AST, U/L	38(25–56)	26(17–42)
HOMA-IR	3.4(2.2–5.7)	3.6(2.2–6.3)
Folic acid, ng/ml	10.2 ± 3.6	10.3 ± 3.9
NAFLD n(%)	62(88.6%)	113(86.9%)

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; TG, triglycerides; TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; ALT, alanine amino transaminase; AST, aspartate amino transaminase; HOMA-IR, Homeostatic model assessment of insulin resistance.

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