Original Article
Validation of the ZJU index for nonalcoholic fatty liver disease in West China: a cross-sectional study

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Abstract: Background: ZJU index, which is an algorithm based on body mass index (BMI), triglycerides (TG), fasting plasma glucose (FPG) and the ratio of serum alanine aminotransferase (ALT) to serum aspartate transaminase (AST), was recently initially developed to detect non-alcoholic fatty liver disease (NAFLD) in the physical examination population of East China. Aim: It aimed to evaluate the accuracy and optimal cut-off point of ZJU index for predicting NAFLD in physical examination population of West China. Methods: This cross-sectional study included 4460 Chinese adults recruited from Sichuan Province Hospital, Chengdu, China. NAFLD was diagnosed by ultrasonography (US). The accuracy and cut-off point of ZJU index to detect NAFLD were evaluated by area under the receiver operator characteristic curve (AUROC). Results: ZJU index predicted the presence of NAFLD with AUROC of 0.873 (95% CI 0.862-0.884). When the cut-off value of ZJU index was chosen at 35.7, the sensitivity was 0.786 and the specificity was 0.799. The US-diagnosed NAFLD individuals presented a higher score of ZJU index when compared with the US-diagnosed non-NAFLD individuals (39.1 ± 4.7 Vs. 32.6 ± 3.9, $P < 0.001$). Conclusions: This study confirmed the accuracy of ZJU index for identifying NAFLD in a large general population in West China. It supported ZJU index as a valuable noninvasive method for the diagnosis of NAFLD with the cut-off point of 35.7.

Keywords: ZJU index, nonalcoholic fatty liver disease (NAFLD), cross-sectional study, West China

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a spectrum of chronic liver diseases, covering from hepatic steatosis to non-alcoholic steatohepatitis (NASH), hepatic fibrosis, cirrhosis and finally to hepatocellular carcinoma (HCC) [1]. The rapidly developing economy worldwide witnessed a phenomenon that sedentary and fast-paced lifestyle led to high pressure and unhealthy food habit. Given the background, the increasing prevalence of obesity, diabetes, and dyslipidemia contributes to the rapid growth in NAFLD [2, 3]. The prevalence of NAFLD was estimated to range from 2.8% to 46% throughout the world and varies depending on the diagnostic methodology and the specific population [4].

In terms of diagnostic standards, liver biopsy is regarded as the most reliable methodology to identify the presence of steatohepatitis, fibrosis and cirrhosis in patients with NAFLD, even though it is widely accepted that biopsy is limited by test cost, sampling error, and medical injure caused by the procedure [5, 6]. Given the nature and epidemiological trend, there has been remarkable interest in identifying clinical prediction standards and non-invasive biomarkers for diagnosis of steatohepatitis in NAFLD patients [7].

Recently, Wang et al. [8] from Zhejiang Univeristy conducted a cross-sectional study with 9602 subjects to develop a simple model to determine the presence of NAFLD, using a stepwise logistic regression analysis to obtain the

model. They named the model as the ZJU index, which was developed based on body mass index (BMI), triglycerides (TG), fasting plasma glucose (FPG) and the ratio of serum alanine aminotransferase (ALT) to serum aspartate transaminase (AST). It found that the area under the receiver operating characteristic curve (AUROC) of the ZJU index to identify NAFLD was 0.822. With a value of ZJU index less than 32, it could rule out NAFLD with a sensitivity of 92.2%, while a value more than 38.0 could identify NAFLD with a specificity of 93.4%. Additionally, to validate this model, a total of 148 patients with liver biopsy found that the ZJU index could distinguish steatosis with an AUROC of 0.896. It suggested that the ZJU index, developed in a Chinese population of east coast, is a helpful model to detect NAFLD for community physicians in China.

Given the significant difference of culture, lifestyle and diet habit between the east coast and the west inland of China, it is essential to validate the ZJU index in a different population [3, 9]. This study is to investigate the accuracy and the optimal cut-off point of the ZJU index for predicting NAFLD in a population of Sichuan, the West China.

Subjects and methods

Subjects

The participants were selected from the health examination programme at Sichuan Province Hospital. This study was approved by the Ethics Committee of Sichuan Province Hospital, in accordance with the Helsinki Declaration of 1975. All subjects gave written informed consent.

Questionnaire

A standard questionnaire was used to collect detailed information on medical history, and lifestyle during a face-to-face interview [9].

Anthropometric examinations

Anthropometric examinations, including height and weight were achieved based on standard procedures as previously described [10, 11]. We calculated Body Mass Index (BMI) based on the following formula: BMI = subject's weight/ height² (kg/m²). Obesity was defined when BMI

was ≥ 25 kg/m², as previously described [10, 11].

Biochemical examinations

All subjects were informed to complete an overnight fast. About 10 ml whole blood samples were collected from every subject, and then serum samples were separated for immediate analysis. The biochemical parameters included: ALT, AST, FPG, and TG.

ZJU index calculation

The formula for the ZJU index calculating is as follow: ZJU index = BMI (kg/m²)+ FPG (mmol/L)+ TG (mmol/L)+3*ALT (IU/L)/AST (IU/L) ratio (+ 2 if female).

Definitions of NAFLD

NAFLD was diagnosed according to the guidelines for diagnosis and treatment of NAFLD issued by Fatty Liver and Alcoholic Liver Disease Study Group of the Chinese Liver Disease Association [12]. It was defined as the ultrasonic presence of fatty liver in the absence of potential causes of the hepatic fat accumulation, including: 1) excessive alcohol intake (> 20 g per day); 2) intake medications that might cause hepatic fat accumulation over the previous 6 months; 3) positivity for hepatitis B virus surface antigen or anti-hepatitis C virus antibody; and 4) other causes. Ultrasonography of the upper abdominal organs was performed by two experienced physicians using a General Electric LOGIQ E9 (General Electric, Fairfield, Connecticut, U.S.).

Statistical methods

Normally distributed variables were presented as mean ± standard deviation (SD); variables with a skewed distribution underwent a lg (x) transformation to achieve a normal distribution and were presented as median value (interquartile range). Normality of distribution was tested with the Kolmogorov-Smirnov test. The Student's t test or Mann-Whitney U test for continuous variables, and χ^2 test or Kruskal-Wallis test for categorical variables were used to compare the parameters between two groups. Comparisons of variables among multiple groups used One-way ANOVA, following with *post hoc* comparisons of LSD. Receiver operating characteristic (ROC) curve of ZJU index was

Table 1. Characteristics of subjects in the study

Variables	Total	Control	NAFLD	*P*
Number	4460	3510	950	
Male (#, %)	2518, 56.5%	1747, 49.8%	771, 81.2%	< 0.001
Age (year)	43.3 ± 13.6	42.7 ± 13.9	45.6 ± 12.1	< 0.001
BMI (kg/m²)	23.3 ± 3.4	22.5 ± 23.0	26.4 ± 2.9	< 0.001
TG (mmol/L)	1.6 ± 1.4	1.3 ± 1.0	2.6 ± 2.1	< 0.001
FPG (mmol/L)	5.0 (4.7-5.4)	5.0 (4.6-5.3)	5.3 (4.8-6.0)	< 0.001
ALT (U/L)	21.0 (14.0-32.0)	18.0 (13.0-27.0)	35.0 (24.0-50.0)	< 0.001
AST (U/L)	24.0 (20.0-29.0)	23.0 (19.0-27.0)	28.0 (23.0-35.0)	< 0.001
ZJU index	34.0 ± 4.9	32.6 ± 3.9	39.1 ± 4.7	< 0.001

Alanine transaminase (ALT), Aspartate transaminase (AST), Fasting plasma glucose (FPG), and Triglycerides (TG). *P* values presented the comparisons between control and NAFLD subjects.
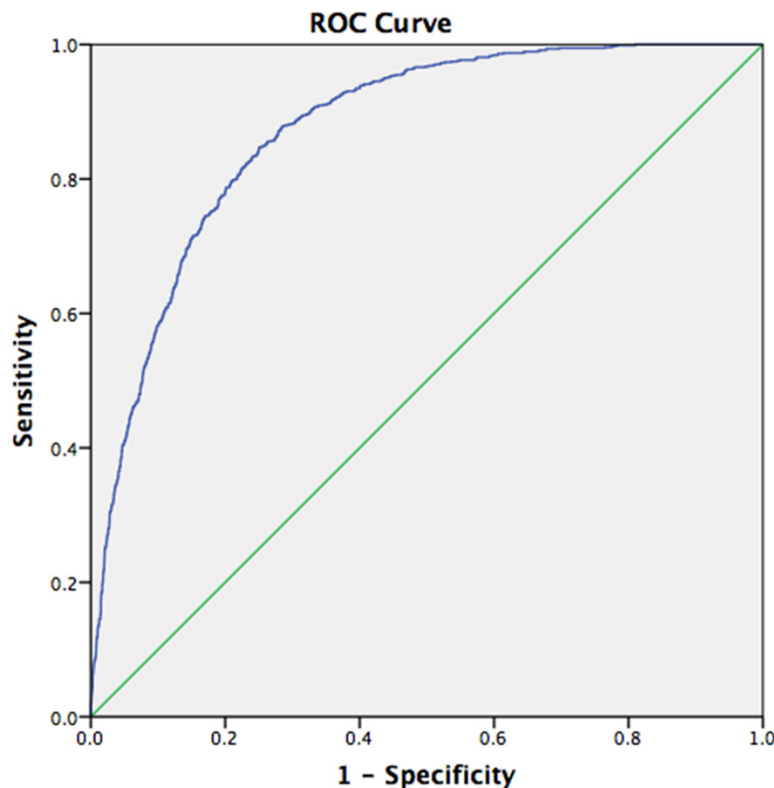

Figure 1. The receive-operating characteristic curve for ZJU index as a diagnostic indicator for nonalcoholic fatty liver disease (NAFLD). Area under the curve = 0.873 (95% CI 0.862-0.884), *P* < 0.001. When the cut-off value chose 35.7, the sensitivity was 0.786 and the specificity was 0.799.

developed to predict the presence of NAFLD. All statistical analyses and plotting were performed using SPSS (version 21; SPSS Inc., Chicago, IL). A two-sided *P* < 0.05 was considered statistically significant.

Results

Characteristics of participants

Table 1 shows the characteristics of the participants in this study. The mean age of all participants was 43.3 ± 13.6 years old, 56.5% were men. The patients with NAFLD were older and had higher levels of BMI, TG, FPG, ALT, AST and ZJU index, in comparison with the non-NAFLD subjects (all *P* < 0.05). The means of the ZJU index in control and NAFLD were 32.6 ± 3.9 and 39.1 ± 4.7, respectively.

The accuracy of ZJU index in NAFLD predicting

ROC curve of ZJU index was used to predict the presence of NAFLD (**Figure 1**). The area under ROC (AUROC) was 0.873 (95% CI 0.862-0.884). When the cut-off value of ZJU index chose 35.7, the sensitivity was 0.786 and the specificity was 0.799.

Comparisons of characteristics between US- and ZJU index-diagnosed NAFLD patients

In total, 950 NAFLD patients and 3510 non-NAFLD participants were defined by US. To be specific, 2810 subjects were defined as control by both ZJU index and US, while 739 subjects were diagnosed as NAFLD patients by both ZJU index and US. As shown in **Table 2**, the variables, e.g. age, BMI, TG, ALT, AST, witnessed significant differences among the four groups, separated by US and ZJU index (all *P* < 0.05).

Table 2. Features of subjects based on ZJU index and US

Variables	ZJU Index (-) US (-)	ZJU Index (-) US (+)	ZJU Index (+) US (-)	ZJU Index (+) US (+)	p
Number	2810	211	700	739	
Male (#, %)	1312, 46.7%	179, 84.9%	435, 62.1%	592, 80.1%	< 0.001
Age (year)	41.5 ± 13.7	46.0 ± 12.6	47.5 ± 13.7	45.5 ± 12.0	< 0.001
BMI (kg/m^2)	21.5 ± 2.2	23.7 ± 1.6	26.3 ± 2.4	27.3 ± 2.7	< 0.001
TG (mmol/L)	1.1 ± 0.6	1.7 ± 0.7	2.1 ± 1.7	2.9 ± 2.3	< 0.001
FPG (mmol/L)	4.9 (4.6-5.2)	4.9 (4.6-5.3)	5.3 (4.9-5.8)	5.4 (4.9-6.2)	< 0.001
ALT (U/L)	17.0 (12.0-24.0)	24.0 (18.0-33.0)	28.0 (20.0-29.0)	39.0 (28.0-54.0)	< 0.001
AST (U/L)	22.0 (19.0-26.0)	25.0 (21.0-30.0)	25.0 (21.0-31.0)	29.0 (24.0-36.0)	< 0.001
ZJU index	31.1 ± 2.5	33.8 ± 1.5	38.5 ± 2.7	40.6 ± 4.2	< 0.001

Alanine transaminase (ALT), Aspartate transaminase (AST), Fasting plasma glucose (FPG), and Triglycerides (TG).

Discussion

The present study found that the ZJU index could detect NAFLD accurately with a good AUROC of 0.873 and the optimal cut-off point of the ZJU index for diagnosing NAFLD was 35.7 with high sensitivity of 78.6% and specificity of 79.9% the southwest Chinese. The ZJU index was suggested to be a valuable noninvasive method for the diagnosis of NAFLD.

Recently, a series of serum biomarkers has been identified for clinical NAFLD diagnosis [13, 14]. To be specific, the NAFLD liver fat score is a system, which depends on five items, i.e. AST, AST/ALT ratio, fasting insulin, the presence of metabolic syndrome and type 2 diabetes [5]. Additionally, the SteatoTest, a six items index, consist of the lipid panel (cholesterol and triglycerides), age, gender, adjusted glucose and BMI [15]. They failed to be widely used, due to limited information while compared with baseline imaging for NAFLD diagnosis [5, 16].

Moreover, recent evidence presented particular interest in CK-18, an intermediate filament protein in the liver, and its role with diagnosis of NAFLD. It found that CK-18 significantly elevated in subjects with NAFLD, especially in patients with biopsy-proven NASH [17]. Unfortunately, the measurement of CK-18 has not been available in clinical practice yet.

Even though other biomarkers, e.g. adipocyte fatty acid binding proteins and fibroblast growth factor 21, had been evaluated in rodent and human studies, less accuracy and instability stop their clinical application [13].

NAFLD is a common cause of chronic liver disease that might progress to hepatic cirrhosis or even hepatic cancer [1]. Liver biopsy, the so-called gold standard for NAFLD diagnosis, unfortunately presents some drawbacks. Currently, no available noninvasive method is clinically ideal. Therefore, there is an urgent need for better diagnostic tools/methodology in clinical practice to evaluate liver injury by hepatic steatosis longitudinally and to monitor the disease progression to fibrosis and cirrhosis.

To the best of our knowledge, this is the first study of large population to validate the accuracy of ZJU index for identifying NAFLD in Sichuan, West China. It supported that ZJU index a valuable noninvasive method for the diagnosis of NAFLD. However, this study has several limitations. To begin with, the diagnosis of NAFLD in this study was based on ultrasound, rather than liver biopsy [18]. Secondly, we failed to have the information of severity of hepatic steatosis, which limited the possibility of indicating specific cutoffs of ZJU index for steatosis quantification.

In summary, it supports that ZJU index might be an economical and noninvasive predictor for the clinical management of NAFLD. We validated the accuracy of ZJU index for identifying NAFLD in a large general population in West China, and detected the optimal cut-off value for NAFLD. To better evaluate the application of ZJU index, it should be additionally validated in one or more representative Chinese populations, e.g. the north China.

Disclosure of conflict of interest

None.

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