Reference Intervals of Fibrosis Index Based on Four Indicators in Healthy Elderly Chinese

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Background: The establishment of reference intervals of total bilirubin, Alanine aminotransferase (ALT), aspartate aminotransferases (AST), and creatinine provided necessary reference in screening and diagnosis of various kidney and liver diseases. However, these reference intervals were not available to estimate liver fibrosis degree. The purpose of this study is to establish the reference intervals of fibrosis index based on the four indicators (FIB-4) in apparently healthy elderly Chinese. **Methods:** A total of 24,949 blood specimens were collected by the standard procedures, and ALT, AST, and PLT were determined. FIB-4 were calcu-

lated by the following formula: $FIB-4 = (age [years] \times AST [U/l]) / ((PLT [10^9/l]) \times (ALT [U/l])^{1/2})$. The elderly's FIB-4 were analyzed between the same age of different sexes and different ages of the same sex. Statistical data were analyzed by SPSS18.0 software. **Results:** Reference intervals of FIB-4 index, established for the healthy elderly, were 0.9923–4.5424 for males and 0.9007–4.1934 for females. **Conclusion:** We established reference intervals of FIB-4 index. This research provided reference value that can be used by relevant clinicians and inspection officers. *J. Clin. Lab. Anal.* **30**: 645–648, 2016. © 2016 Wiley Periodicals, Inc.

Key words: liver fibrosis; FIB-4 index; reference intervals

INTRODUCTION

Estimating the degree of liver fibrosis is significantly important for the clinical progress of viral hepatitis' (hepatitis B and hepatitis C, etc.) development, the choice of antiviral drugs, curative effect evaluation, and prognosis evaluation of patients. Liver biopsy, which is regarded as the gold standard in the assessment of severity of liver disease, is usually used for assessing the level of necroinflammatory activity and fibrosis, and to diagnose cirrhosis in chronic viral hepatitis. However, the liver biopsy is an invasive examination that could induce traumatic complications such as bleeding, pain, and pneumothorax when the operation lacks standardization (1, 2). Meanwhile, liver biopsy strongly influences the grading and staging of chronic viral hepatitis (3). As a result, most patients are unwilling to accept liver biopsy, which is the limitation of the clinical application of liver biopsy. Therefore, it is difficult to observe the progress of the liver disease dynamically. For the reasons above, it is necessary to develop a simple, accurate, applicable, low-cost, and noninvasive liver fibrosis examination method in order to observe the changes of hepatic fibrosis preferably. In

recent years, researchers around the world are working with noninvasive detection system that assesses the degree of liver fibrosis mainly by biological and imaging studies, but there are some limitations in terms of accuracy and sensitivity of this detection system (4, 5). Several studies have shown that most noninvasive prediction models such as FibroMeter A, HeapScore, and FibroTest could predict negative predictive value to eliminate significant fibrosis preferably (6–8). FIB-4 index (where FIB is fibrosis index based on the four indicators) was proposed to assess liver fiber in patients with HIV/HCV superinfection, and this index achieved excellent results that made 71% of the patients avoiding unnecessary liver puncture biopsy (9).

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FIB-4 index ($\text{FIB-4} = (\text{age} [\text{years}] \times \text{AST} [\text{U/L}]) / ((\text{PLT} [10^9/\text{L}] \times (\text{ALT} [\text{U/L}]^{1/2}))$), which included three clinical indicators ALT, AST, and PLT, was considered to be simple, accurate, and economic evaluation index (9, 10). However, there was no a unified reference standard (reference intervals) or baseline when evaluating the degree of liver fibrosis using the noninvasive index (FIB-4 index). So, it is necessary to establish reference intervals (RIs) of the FIB-4 index.

MATERIALS AND METHODS

Ethics Approval

This study was approved by the ethics committee of the Shuyang People's Hospital.

Subject Selection

A total 100,000 elderly Chinese of Han ethnicity participated in physical examination organized by Shuyang County government and Shuyang People's Hospital between January 2012 and December 2012. All participants signed a written informed consent and filled in the questionnaires according to the Clinical and Laboratory Standards Institute (CLSI) C28-A3 document recommendations (11).

For the determination of biochemical parameters, all subjects were required to maintain their normal lifestyle, avoid strenuous physical exercise within 3 days of the physical examination and laboratory testing, and refrain from drinking alcoholic beverages for at least 1 day prior to the testing. The subjects fasted overnight (fasting time was at least 8 hr) and sat for at least 30 min before specimen collection. Approximately 5 ml of blood was collected in a separation gel vacuum tube (KEHUA Biosciences, Ltd., Shanghai, China) between 7:30 am and 10:30 am. The samples were kept at room temperature for 30 min followed by centrifugation for 10 min at 4,000 rpm. All of the analyses were performed within 8 hour after serum collection. The PLT, ALT, and AST values were measured by the Sysmex XE-2100 automated hematology analyzer (Sysmex Co., Ltd., Japan) and a TBA2000FR automatic biochemical analyzer (Toshiba Co., Ltd., Japan) on standardization of the laboratories. The ALT and AST reagents were obtained from KEHUA Biosciences (UV-MDH Method, Liquid, without pyridoxal phosphate).

To minimize errors, the tests were performed according to the standard operating procedures. The performance of the assays was reflected by the standard deviation and coefficient of variation (CV) derived from the observed means, which were interpreted based on the Westgard rules.

Data Exclusion and Instrument Quality Control

We excluded PLT data of patients who had hematological system diseases and of patients whose blood analysis provided alarming information or histogram was abnormal. AST data were also excluded for the patients with hepatopathy (such as positive HBV surface-antigen) and heart disease. We also excluded data from the hemolysis specimens.

The Sysmex XE-2100 automated hematology analyzer and TBA2000FR automatic biochemical analyzer were adjusted after being installed. The reagents and standard substance used in these two instruments were from Japan original and KEHUA Biosciences, Ltd., respectively. Bio-Rad Ltd: 14411, 14412, and Randox Ltd.: UN1557-639UN, respectively, evaluated the internal quality control of the two instruments.

FIB-4 Index Measurement

After measuring PLT, AST, and ALT, we calculated FIB-4 index according to the following formula: $\text{FIB-4} = (\text{age} [\text{years}] \times \text{AST} [\text{U/L}]) / ((\text{PLT} [10^9/\text{L}] \times (\text{ALT} [\text{U/L}]^{1/2}))$.

Statistical Analysis

Data were pooled and analyzed using nonparametric method (2.5th–97.5th percentiles) and 95% reference intervals according to the CLSI C28-A3 guideline (13, 14). For outlier exclusion, Dixon-Reed's outlier method was used. Possible partitioning of two broad age groups, 65–74 and 75 years, was done using the robust method with outliers detected by visual inspection of distributions and Tukey's criterion. Mann–Whitney *U*-test was used for determining FIB-4 index of significantly different age groups. Statistical analyses were performed by SPSS 18.0.

RESULTS

We selected 24,949 healthy individuals aged 65–104 (7,102 males and 17,847 females) as our subject of 100,000 participants after X-ray, electrocardiogram, liver and kidney function tests, and a questionnaire (about lifestyle, including dietary habits, smoking and drinking status, hypertension, diabetes, and recent drug using) (11, 12). The sample size of this study was in accordance with the rigorous CLSI guidelines for determining laboratory reference ranges, which recommends a minimum of 120 subjects for a 95th percentile clinical reference range determination with 95% confidence intervals for each partition group (15). All subjects were free of diseases of the blood system, digestive system, chronic kidney disease, and other chronic diseases. Table 1 shows that the hematological

TABLE 1. Comparison of Hematological and Biochemical Parameters Between Males and Females

Items	Males (mean and IQR ^a)	Females (mean and IQR)	P-value
Number	7,102	17,847	
Age (years)	73 (68–80)	75 (69–84)	0.009
PLT ($\times 10^9/l$)	215 (182–268)	219 (183–279)	<0.001
ALT (U/l)	18 (14–45)	19 (18–37)	0.004
AST (U/l)	26 (20–49)	24 (21–45)	<0.001

^aIQR, interquartile range.

and biochemical parameters of ALT, AST, and PLT were statistically significantly different between genders of each age group.

The critical value was $Z > Z^*$ for the FIB-4 index level. On calculation of the sex-specific RIs for FIB-4 index ($Z > Z^*$), it was observed that FIB-4 index level was higher for men than women. As age is an essential component in calculating FIB-4, it is reasonable that FIB-4 is affected by age. Finally, the participants were divided into two groups according to gender.

Figure 1 shows the histograms of different ages and gender for FIB-4. A nonparametric approach was used to construct the reference values, and separate RIs were established for these two age groups. The 2.5 and 97.5 percentile of the FIB-4 index level and their respective 95% confidence intervals are shown in Table 2. Reference intervals of FIB-4 index established for the healthy elderly were 0.9923–4.5424 for males and 0.9007–4.1934 for females. There was no statistically significant difference ($P > 0.05$) between two groups.

DISCUSSION

Estimating the degree of liver fibrosis is extremely important for the development of viral hepatitis, the choice of antiviral drugs, curative effect evaluation, and

prognosis evaluation. This study established RIs for FIB-4 providing reference to the clinical physician. Moreover, WHO issued its first hepatitis B treatment guidelines, in which simple noninvasive tests (NITs) were recommended to assess the stage of liver disease for appropriate treatment. FIB-4 were recommended as the preferred NIT to assess the presence of cirrhosis ($\text{FIB-4} > 3.25$) in resource-limited settings.

Previous studies did not report FIB-4 index as a reference interval for lacking relevant reference standard with different individual baseline levels. FIB-4 reference intervals could provide significant guiding function for individualized treatment. Our research established FIB-4 indexes of 0.9923–4.5424 for males and 0.9007–4.1934 for females for healthy elderly Chinese in Shuyang county. Furthermore, application of FIB-4 indexes would be generalized to other districts in China, especially for the healthy elderly.

Although we established FIB-4 RIs in China for the first time, there are some limitations. First, subjects we selected were the elderly who might not represent all healthy adults; however, we did not believe this was a big problem in this study because the subjects were free from relevant diseases. Second, establishment of FIB-4 index was based on other four indicators: age, PLT, AST, and ALT that might affect accuracy of FIB-4 index for performance of different analyzer instruments. Third, physical examination season for different subjects would be a factor of time bias. Fourth, some confounders, such as BMI, drugs of taking hepatinica, were not controlled in our study, which would affect examination values.

In summary, our results support the hypothesis of establishing the FIB-4 index reference intervals for the healthy Chinese, especially for the elderly. Further, studies are still needed to evaluate the effects of FIB-4 index on estimating liver fibrosis degree, and to elucidate the potential roles of abnormal FIB-4 index in the pathophysiology of hepatopathy and other system diseases.

Fig. 1. The histograms of different ages and different gender for FIB-4.

TABLE 2. Ninety-Five Percent Reference Intervals for FIB-4 in Healthy Elderly^a

Groups	Gender ^b	Number	FIB-4 index				
			Median	Lower limit	90% CI	Upper limit	90% CI
Total		24,949	1.9109	0.9241	0.9137–0.9324	4.3203	4.2662–4.4020
	Male	7,102	2.0434	0.9923	0.9751–1.0156	4.5424	4.4430–4.6321
	Female	17,847	1.8627	0.9007	0.8910–0.9097	4.1934	4.1475–4.2756

^a Calculated by nonparametric percentile method (2.5th–97.5th percentiles) and 95% reference interval, double-sided according to the CLSI C28-A3 guideline.

^b Mann–Whitney *U*-test for differences between males and females.

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CONFLICT OF INTEREST

No authors declared any potential conflicts of interest.

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