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Reference Intervals of Total Bilirubin, ALT, AST, and Creatinine in Healthy Elderly Chinese

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Statistical Analysis C
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Background: The aim of this study was to establish the reference intervals (RIs) of total bilirubin (TBIL), alanine aminotransferase (ALT), aspartate transaminase (AST), and creatinine (CREA) for apparently healthy elderly (Han ethnicity) in Shuyang, China.

Material/Methods: A total of 54 912 blood specimens from elderly residents age 65–104 years were collected by standard procedures in Shuyang county of Jiangsu province. TBIL, ALT, AST, and CREA for each participant were determined by automatic biochemical analyzer. Distribution and differences of TBIL, ALT, AST, and CREA were analyzed and compared between the elderly of the same age of different sexes and different ages of the same sex. RIs of TBIL, ALT, AST, and CREA were compared with the current RIs. The RIs and 95% confidence intervals were calculated using nonparametric method (2.5^{th}–97.5^{th} percentiles) according to the guideline of the Clinical and Laboratory Standards Institute.

Results: RIs established for the healthy elderly include: TBIL 7.8~30.6 μmol/L for males and 7.3~26.1 μmol/L for females; ALT 8.7~47.3 U/L for males and 8.4~45.2 U/L for females; AST 15.7~46.9 U/L for males and 15.1~46.2 U/L for females; and CREA 45.1~100.9 μmol/L for males and 38.7~85.0 μmol/L for females. Reference intervals of TBIL, ALT, AST, and CREA for male elderly were higher than those of females, and values of CREA increased with increasing age.

Conclusions: We have established a panel of locally relevant RIs. It is necessary to establish scientific and reasonable RIs of TBIL, ALT, AST, and CREA for the healthy elderly in our region, which will provide a reference for clinicians and inspection officers.

MeSH Keywords: Alanine Transaminase • Creatinine • Reference Values

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Background

Reference intervals (RIs) are essential for clinical diagnosis, treatment, and management. However, RIs of test items being used in most Chinese laboratories have been obtained from RIs data of foreign populations before the implementation of the new national standard. Currently, there are no systematic RIs for Chinese elderly, restricted by testing technique, economic level, and other factors [1]. TBIL, ALT, AST, and CREA are important clinical indicators, especially in terms of screening and diagnosis of various kidney and liver diseases [2,3].

Many clinical indicators of the elderly are different from those of younger adults, so it is necessary to establish RIs of TBIL, ALT, AST, and CREA for the elderly populations in our region to offer better guidance of medical diagnosis and treatment of diseases for the elderly. RIs for the young are widely used internationally, but there appear to be no RI for the elderly on an international basis.

Material and Methods

Ethics approval

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

Subject selection

We selected 54 912 healthy Chinese Han ethnicity elderly individuals (aged 65–104 year) out of 100 000 participants between January 2012 and December 2012. There were 35 802 (65.2%) females and the mean age was 74 years old. As described previously [2,4], a questionnaire (including lifestyle, including dietary habits, drinking, smoking status, hypertension, diabetes, and recent drug use) was used and chest X-ray, electrocardiogram, and liver and kidney function were screened for all participants, and the results of these were normal. All subjects were free of diseases of the blood system, digestive system, chronic kidney disease, and other chronic diseases. They did not drink alcohol within the past 3 days and did not smoke within the past 3 hours. All participants signed a written informed consent and filled in the questionnaires according to the CLSI C28-A3 document recommendations [3].

Laboratory methods

All of the candidate reference individuals were required to maintain their normal lifestyle and avoid strenuous physical exercise within 3 days of the physical examination and laboratory testing; they were also required to refrain from drinking alcoholic beverages for at least 1 day prior to the testing. The candidate reference individuals fasted overnight (fasting time was at least 8 h) 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, 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 4000 rpm. All of the analyses were performed within 8 h after serum collection. The TBIL, ALT, AST, and CREA values were measured by a TBA2000FR automatic biochemical analyzer (Toshiba Co., Ltd., Japan) on standardization of the laboratories.

To minimize errors, the tests were performed according to 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.

Statistical analyses

Data were pooled and analyzed using nonparametric method (2.5^{th}–97.5^{th} percentiles) and 95% reference intervals according to the CLSI C28-A3 guideline [3,5–7]. Gaussian distribution of all data was tested by the method of the Kolmogorov-Smirnov. For outlier exclusion, Dixon-Reed's outlier method was used. Possible partitioning of 2 broad age groups: 65–74 years and ≥75 years were done using the robust method with outliers detected by visual inspection of distributions and Tukey's criterion. Mann-Whitney U test was used to determine TBIL, ALT, AST, and CREA of significantly age-different groups. Statistical analyses were performed by MedCalc software (version 11.4.2.2, Ostend, Belgium) and SPSS 18.0.

Results

After screening the original subjects and eliminating any outliers, the current study included a total of 54 912 subjects (19110 males and 35802 females). 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 95^{th} percentile clinical reference range determination with 95% confidence intervals for each partition group [3]. Table 1 shows the main characteristics of the participants in this study.

A one-sample Kolmogorov-Smirnov test of abnormality indicated that the distribution of TBIL, ALT, AST and CREA approximated a non-Gaussian distribution ($P<0.05$). The critical value was $Z>Z^*$ for the TBIL, ALT, AST, and CREA levels. Upon calculation of the sex-specific RIs for TBIL, ALT, AST, and CREA ($Z>Z^*$), it was observed that the men's TBIL, ALT, AST, and CREA levels were higher than the women's. Selected participants

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Table 1. Baseline characteristics of the participants by sex (mean ± standard).

	Male			Female		
	Total	65–74 years	75-years	Total	65–74 years	75-years
N	19110	12467	6643	35802	21658	14144
Fasting glucose (mmol/L)	5.11±0.61	5.08±0.59	5.29±0.65	5.23±0.59	5.17±0.56	5.35±0.62
Total cholesterol (mmol/L)	4.78±0.87	4.91±0.85	4.73±0.88	5.19±0.95	5.16±0.92	5.25±0.98
Triglyceride (mmol/L)	1.26±0.45	1.26±0.43	1.27±0.49	1.32±0.52	1.32±0.51	1.33±0.53
Hemoglobin (g/L)	142.8±13.6	144.9±11.1	131.7±10.8	130.5±11.8	142.9±13.4	127.2±10.9

Table 2. The sex- and age-dependent RIs of the TBIL, ALT, AST, and CREA levels in the elderly population of China*.

Age group (years)	sex	Case number	TBIL (μmol/L)		ALT (U/L 37°C)		AST (U/L 37°C)		CREA (μmol/L)	
			Med	$P_{2.5}$~$P_{97.5}$	Med	$P_{2.5}$~$P_{97.5}$	Med	$P_{2.5}$~$P_{97.5}$	Med	$P_{2.5}$~$P_{97.5}$
65–74	Male	12467	15.0	8.0~30.3	18.9	8.8~48.5	26.0	16.1~46.3	65.0	58.0~97.0
	Female	21658	13.5	7.5~25.8	17.6	8.4~45.5	24.8	15.4~45.6	53.0	38.1~83.1
Total		34125	14.1	7.7~28.0	18.4	8.5~47.6	25.2	15.8~46.1	56.7	39.0~90.0
>74	Male	6643	14.8	7.4~31.4	18.6	8.6~44.8	25.6	15.2~47.7	68.0	46.0~104.2*
	Female	14144	13.4	6.9~26.6	17.7	8.5~44.7	24.4	14.8~46.9	54.9	39.1~87.0*
Total		20787	13.8	7.0~28.5	17.8	8.5~44.7	24.8	14.9~47.2	58.1	40.1~96.0
Older age-group	Male	19179	14.9	7.8~30.6	18.7	8.7~47.3	25.8	15.7~46.9	66.0	45.1~100.9
	Female	35733	13.5	7.3~26.1	17.6	8.4~45.2	24.7	15.1~46.2	53.9	38.7~85.0

* Calculated by method (2.5^{th}–97.5^{th} percentiles) by CLSI C28-A3 guideline.

Table 3. Comparisons of RIs for TBIL, ALT, AST, and CREA between China and other countries.

items groups		Current study			Current standard	USA [21]	Tanzania [22]
		65~74	>74	65~			
TBIL (μmol/L)	Male	8.0~30.3	7.4~31.4	7.8~30.6	5.1~19 [5]	5.1~17.0	5.2~41.0
	Female	7.5~25.8	6.9~26.6	7.3~26.1	5.1~19 [5]	5.1~17.0	5.2~41.0
ALT (U/L 37°C)	Male	8.8~48.5	8.6~44.8	8.7~47.3	9~50 [1]	0~35	9~55
	Female	8.4~45.5	8.5~44.7	8.4~45.2	7~40 [1]	0~35	7~45
AST (U/L 37°C)	Male	16.1~46.3	15.2~47.7	15.7~46.9	15~40 [1]	0~35	15~53
	Female	15.4~45.6	14.8~46.9	15.1~46.2	13~35	0~35	14~35
CREA (μmol/L)	Male	58.0~97.0	46.0~104.2	45.1~100.9	59~104 [5]	<133	48~96
	Female	38.1~83.1	39.1~87.0	38.7~85.0	45~84 [5]	<133	40~81

were divided into different groups by every 10 years for an age group. TBIL, ALT, AST, and CREA values did not show statistically significant difference (P>0.05) among the 3 groups (75–84, 85–94, and 95–104 years) in both sexes in our study. Therefore, these 3 groups were combined into 1 age group (75–104 years). Finally, the candidate participants were divided into 2 groups (65–74 and 75–104 years) according to age in each sex. A significant difference was observed among the individuals aged 65–74 and 75–104 in terms of sex (P<0.05). A non-parametric approach was used to construct the reference values, and separate RIs were established for these 2 age groups. The 2.5 percentile and 97.5 percentile of the TBIL, ALT, AST, and CREA levels and their respective 95% confidence intervals are shown in Table 2.

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When candidate participants were divided into different groups by every 5 years for an age group, TBIL, ALT, AST, and CREA values did not show statistically significant difference (P>0.05) between males and females for each group (65–69, 70–74, 75–79, 80–84, and >84 years) in our study, but TBIL, ALT, AST, and CREA values showed statistically significant difference (P<0.05) among these 5 groups for the same sex, and CREA values increased with increasing age (Table 3).

Discussion

The World Health Organization (WHO) defines a person who is more than 65 years old as elderly. This guideline is adopted by Europe, the United States, and other developed countries, whereas in the Asia-Pacific region, the age defined as "elderly" is more than 60 years old [8]. According to the statistics of the sixth national population census from the National Bureau of Statistics of China in 2010, nearly 11% of the population (equivalent to 143 million) in China was classified as elderly [9].

Currently, most of the clinical laboratories in China (even the large ones) either use the RIs provided by the assay manufacturers or rely on foreign population data, focusing specifically on Caucasians [10,11]. Adult reference values have been well established in the past few years [12–19]. However, for the elderly population, the RIs of many analytes and analytical methods were either inadequate or unavailable. Correctly established population-based RIs are valuable references [11,19]; thus, we believe that it is necessary to establish appropriate RIs for our geriatric population according to their defining characteristics.

In this study, we employed a nonparametric statistical method to calculate the central 95% (between the 2.5th and the 97.5th percentiles) percentile of TBIL, ALT, AST, and CREA levels as RIs in the healthy elderly Chinese population. The results of our study confirm both sex- and age-related changes of the TBIL, ALT, AST, and CREA values.

TBIL of healthy individuals mainly exist in the aging red blood cells, and is in a constant level in normal condition. In the past it was thought that bilirubin was toxic, and a patient with a high TBIL test result but without clinical symptoms was suggested to have periodic testing or to stop the bilirubin treatment. Recent studies showed that bilirubin has an antioxidant effect, and therefore a high level was beneficial [20]. The results of this survey show that TBIL serum level of healthy elderly people is higher than that of the current Chinese reference range and the United States [21], but lower than that of Tanzania [22]. TBIL RIs for males and females is 7.8~30.6μmol/L and 7.3~26.1 μmol/L, respectively, which is consistent with that reported by Liu et al. [23], but significant differences exist between men and women and men's is higher than women's, which is different from previous reports [21,22]. High bilirubin level may be associated with the raising of living standards, living and eating habits, nutrient intake, and increased hemoglobin caused by menopause.

ALT and AST are important indicators of liver function. Our study results show that ALT and AST need to be grouped by sex when detected [24]. ALT reference intervals for males and females are 8.7~47.3 μmol/L and 8.4~45.2 μmol/L respectively, which are close to the new standard [1] but higher than that of the U.S. and Tanzania [22]. The above grouping method of these 3 indicators of liver function is consistent with previous reports [25].

CREA, which is a terminal metabolite of creatine, is a very useful indicator of kidney function. CREA is not sensitive to early renal function decrease, but is extremely important for the staging of renal function. CREA RIs for males and females are 45.1~100.9 μmol/L and 38.7~85.0 μmol/L, respectively, which were close to the new standard [1] and that of Tanzania [22], but lower than that of the U.S. [21].This study shows that CREA levels rise with increasing age, and that men's was obviously higher than women's, the reason for which might be that CREA was related to the total muscle mass in the body. CREA RIs of males and females for the different age groups (65–69 years, 70–74 years, 75–79 years, 80–84 years, and >84 years) were 44.5~95.9 μmol/L and 38.0~82.9 μmol/L, 45.6~98.7 μmol/L and 38.6~84.2 μmol/L, 45.8~103.9 μmol/L and 39.0~87.3μmol/L, 46.7~105.2 μmol/L and 39.0~87.3 μmol/L, and 46.4~105.7 μmol/L and 40.0~90.9 μmol/L, respectively.

The current RIs for these 4 indicators have the following advantages compared with previous research: 1) Large study sample sizes. All samples were from healthy elderly in the region, and were similar, with a random sample selection, and reduced sampling error; 2) Rigorous screening for the healthy population. A comprehensive questionnaire survey and laboratory inspection of this study eliminated factors influencing these 4 indicators; 3) Comprehensive quality control. The related quality controls were done comprehensively and data were used after completing all questionnaires and all relevant laboratory examinations.

Without doubt, this survey also has some limitations, mainly: 1) This investigation was confined to Shuyang County, Jiangsu Province, and the results might not be useful in other areas; 2) Reagents used in this study were from the same company, and the methods for these 4 indicators are the same.

Conclusions

It is necessary to establish the RIs of the elderly for TBIL, ALT, and AST by sex, but not by age; CREA RIs of the elderly should be established according to different age and sex groups.

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Declaration of interest

The authors declare no conflict of interest.

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