Establishment of Reference Intervals For Serum Liver Function Tests Among Healthy Elderly Population In Northeast China: A Retrospective Study

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Research Article

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Abstract

**Background:** Liver function changes with age, however, there are few studies that are specific for the elderly. This study is aimed to establish reference intervals (RIs) of serum liver function tests among healthy elderly population aged between 60-89 in northeast China.

**Methods:** Subjects were collected from laboratory information system (LIS) in the First Hospital of Jilin University. The following parameters were collected: aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP), total protein (TP) albumin (ALB), total bilirubin (TBIL), and direct bilirubin (DBIL). Tukey method was used to eliminate outliers. The Harris and Boyd method and Mann-Whitney U-test were performed to evaluate significant differences between subgroups of sex and age. The lambda-mu-sigma (LMS) method was used to analyze the dynamic changes of analytes. RIs were established by the non-parametric method.

**Results:** A total of 23597 healthy individuals, including 20048 subjects (18-59 years old) and 3549 subjects (60-89 years old) were enrolled in the study. AST, ALT, TP and ALB required no sex partition. Except for AST, ALP and TBIL, ALT, GGT, TP, ALB and DBIL required different levels of age partitions. Serum ALT and ALB levels decreased with age, ALB showed apparent decreases throughout the aging process. DBIL showed an increase trend over time. This study showed different results compared with RIs in other studies.

**Conclusions:** The RIs for liver function tests among healthy elderly population were different from those of other young individuals. There were apparent sex or age differences in the RIs of liver function for the elderly. Therefore, it is necessary to establish sex- and age-specific RIs of serum liver function tests among elderly population.

**Background**

Biological reference intervals (RIs) are an important basis for clinicians to interpret patients’ test results, which are usually defined as the 95 percentile central distribution of apparently healthy individuals. Nowadays, the RIs used in laboratories are mostly originated from the manufacturer or studies on European and American populations a dozen years ago. However, these RIs are not always appeal to the characteristics of all the population. Some RIs are often derived from very small sample sizes, well below recommendations of the International Federation of Clinical Chemistry (IFCC). Furthermore, inappropriate RIs used by laboratories could potentially lead to misdiagnosis, inaccurate therapeutic approaches and duplicate detection. Hence, each laboratory should establish its own RIs according to defined procedures[1–2].

According to the 7th nation-wide census of Chinese population in 2020, China had 264.02 million people aged ≥ 60 years, accounting for 18.7% of the total population (Available from: http://www.zhujia120.com/life/337677.html). With the increasing trend of aged population in China, the demands for medical services are increasing and the aged population seek medical security more often
than young individuals. Unfortunately, the reference population is usually made up of young individuals. Many biomarkers change with age, and RIs based on a population between 18-59 years old may thus not be suitable for people aged \( \geq 60 \) years old. Compared with young individuals, the elderly are not only in poor physical function and nutritional status, but also in higher risk of chronic diseases such as hypertension and diabetes\(^3\). Some routine clinical chemistry parameters showed wider RIs for the elderly\(^4\). For a long time, RIs for young individuals have been using in the elderly, which brings clinicians a lot of confusion. Thus, there is a need to establish RIs for the elderly.

The liver is not only the largest digestive organ in human body, but also an important detoxification organ\(^5\). Biochemical test of liver function is a routine test item for the assessment of liver status\(^6\). Important parameters such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP), total protein (TP) albumin (ALB), total bilirubin (TBIL), and direct bilirubin (DBIL), which play a suggestive role in the early stage of hepatobiliary diseases. Currently, there have been studies on the RIs of liver function in the elderly among other countries\(^7\)–\(^8\). In China, a multi-center study on the RIs of liver function based on a large healthy adult population also can be found\(^9\). However, there are few studies about liver function tests’ RIs that are specific for Chinese elderly population.

Therefore, this study aimed to establish sex- and age-specific RIs for serum liver function tests in elderly population, with the methods and guidelines proposed by the Clinical Laboratory Standards Institute (CLSI). And compare the RIs against various sources. It can be helpful to provide a theoretical basis for clinicians to correctly evaluate the liver function of the elderly.

**Methods**

**Study participants**

We conducted a retrospective study focusing on the liver function in the elderly. The data were collected from the Laboratory Information System (LIS) of the First Hospital of Jilin University from April 2020 to April 2021. Exclusion and inclusion criteria were performed as shown in Figure 1. Finally, 23597 subjects were enrolled in this study, including 20048 subjects (18-59 years old) and 3549 subjects (60-89 years old).

This study was approved by the Ethics Committee of the First Hospital of Jilin University (NO:2021-543) and was conducted in accordance with the Declaration of Helsinki.

**Sample collection**

All participants should fast for 12 hours over night and avoid alcohol in-taking during the last 24 hours before blood collection. Blood samples (4ml) were collected into serum separation tubes (SST™; BD). After storing at room temperature (approximately 22°C) for 30 minutes, samples were centrifuged at
It is worth noting that samples with hemolysis, lipidemia, or jaundice were excluded from further study.

**Laboratory methods and quality control**

All biochemical parameters were analyzed by Hitachi 7600-210 automatic analyzer (Hitachi High-Technologies, Tokyo, Japan). The measured parameters and methods were shown as following: AST (UV-MDH method), ALT (UV-LDH method), GGT (L-γ-glutamyl-3-carboxy-4-nitranilide method), ALP (AMP buffer method), TP (biuret reaction), ALB (bromocresol green method), TBIL and DBIL (DCA method). In 2012, the Department of Laboratory Medicine of the First Hospital of Jilin University accredited ISO 15189 Medical Laboratories-Particular Requirements for Quality and Competence by the China National Accreditation Service for Conformity Assessment. Preventive maintenance was carried out regularly to monitor the performance of biochemical analyzer according to the manufacturer's regulations. All samples were tested within normal quality control.

**Statistical analysis**

Data were analyzed using Excel 2007, SPSS 26.0 software, Minitab version 19.0, GraphPad Prism 8.0 and LMS chartmaker Light 2.54. Kolmogorov-Smirnov test was performed to access the distribution of data. If the data showed a non-Gaussian distribution, Box-Cox method was used to transform the data to approximately Gaussian distribution. Tukey method was used to eliminate outliers[10]. After calculating the values of 25th and 75th percentiles of data distribution (P_{25}, P_{75}) and IQR (inter-quartile range), the lower and upper limits were calculated as P_{25}−1.5*IQR and P_{75}+1.5*IQR. Any data out of this range should be regarded as outliers. The Harris and Boyd method was used to determine whether it was necessary to partition the reference values for sex[11]. The difference between two age groups in subjects was compared using the Mann-Whitney U-test. P-values < 0.05 were considered statistically significant. The lambda-mu-sigma (LMS) method was used to analyze the dynamic changes of analytes. The lower limits (LL) and upper limits (UL) of RIs (2.5th and 97.5th percentiles) were calculated by the non-parametric method.

**Results**

**Experimental population**

A total of 27670 subjects were collected from the LIS. Of these, 1968 data were excluded either for duplicate data or exceeding twice than the limits of national standard. Except for TP and ALB, other analytes showed skew distribution, and were transformed into approximately Guassian distribution by Box-Cox method. Besides, 2100 data were eliminated by Tukey method. Finally, 23597 subjects met the inclusion criteria, and the ratio of male to female is 1:1.18. Each subgroup comprised as the following: men < 60 years old, \( n = 8996 \) and mean age = 43.0±9.8 years); men \( \geq 60 \) years old, \( n = 1820 \) and mean age = 66.8±6.0 years); women < 60 years old, \( n = 11052 \) and mean age = 40.3±9.9 years); women \( \geq 60 \) years old, \( n = 18093 \) and mean age = 66.8±6.0 years).
years old, \((n = 1729\) and mean age = 67.1±6.0 years). The concrete process was demonstrated in Figure 1.

**Comparison of analytes between the young and the elderly**

The median and RIs for each analyte in the \(< 60\) and \(\geq 60\) years old groups divided by sex are summarized in Table 1. Except for AST and ALP, a statistical difference in the medians of other analytes were observed between these two age groups: concentrations of ALP, TBIL, and DBIL in males \(\geq 60\) years were higher than those aged \(< 60\) years, while concentrations of AST, ALT, GGT, TP, ALB in males aged \(< 60\) years were higher. AST, ALT, GGT, ALP, TP and TBIL levels in females \(\geq 60\) years were higher than those aged \(< 60\) years, whereas serum ALB and DBIL levels showed the opposite results.

**Table 1** Sex- and age-specific medians and RIs for 8 liver function parameters of healthy adults

| Parameters | Reference Intervals | Median [P_{2.5} - P_{97.5}] |
|------------|---------------------|-----------------------------|
| AST        | [41.7-115.8] U/L    | 22.5 [9.1-81.5] U/L         |
| ALT        | [8.0-27.1] µmol/L   | 17.3 [8.9-35.7] µmol/L      |
| GGT        | [1.6-7.9] µmol/L    | 3.6 [1.6-7.9] µmol/L        |
| ALP        | [41.7-115.8] U/L    | 16.4 [7.2-61.6] U/L         |
| TP         | [8.0-27.1] µmol/L   | 14.1 [8.0-27.1] µmol/L      |
| ALB        | [1.1-5.2] µmol/L    | 2.3 [1.1-5.2] µmol/L        |
| TBIL       | [38.3-99.3] µmol/L  | 3.6 [1.6-7.9] µmol/L        |
| DBIL       | [1.1-5.2] µmol/L    | 2.3 [1.1-5.2] µmol/L        |

**Sex-specific distributions of liver function tests in the elderly**

The study revealed a strong sex-dependency in the RIs of GGT, TBIL, and DBIL. The median, 2.5th and 97.5th percentiles \((\text{median} [\text{P}_{2.5} - \text{P}_{97.5}]\) of GGT, TBIL and DBIL in males: \((22.5 [9.1-81.5] \text{ U/L}, 17.3 [8.9-35.7] \text{ µmol/L}, 3.6 [1.6-7.9] \text{ µmol/L, respectively}) were significantly higher than those in females \((16.4 [7.2-61.6] \text{ U/L}, 14.1 [8.0-27.1] \text{ µmol/L}, 2.3 [1.1-5.2] \text{ µmol/L, respectively})\). Whereas ALP appeared to be opposite, whose values were significantly higher in females than those in males \((69.3 [41.7-115.8] \text{ U/L vs. 60.5 [38.3-99.3] U/L})\). There was no significant sex difference in the remaining analytes. Sex-specific distributions of liver function tests in the elderly are shown in Figure 2.

**Age-specific distributions of liver function tests in the elderly**

All subjects were partitioned according to age, with group intervals of 10 years \((\text{males/females of 60-69 years old: n=1373/1254, 70-79 years old: n=352/380, 80-89 years old: n=95/95})\). There was no significant difference among age subgroups for AST, GGT in females, ALP and TBIL \((P>0.05)\). Hence, age partitions were not required for these analytes.

The percentile curves of each analyte are shown in Figure 3. The trend of all analytes should be interpreted by the 50th percentile curve \((\text{P}_{50})\) in the figure, because it is the most stable and representative. ALT levels decreased throughout the aging process and ALB showed an apparent decrease with age. GGT levels also showed a downward trend with age in males. Concentrations of TP decreased slightly with age. On the contrary, DBIL levels increased with age in males and females. The median levels of AST, ALP, TBIL remained relatively stable throughout the age range.
| Age group (years old) | n     | Median | RIs    | Lower 90% CI | Higher 90% CI | P-value (age) |
|-----------------------|-------|--------|--------|--------------|---------------|---------------|
| **Male**              |       |        |        |              |               |               |
| AST U/L               | 18-59 | 8996   | 20.5   | 13.4-38.4    | 13.3-13.6     | 37.7-39.2     |
|                       | ≥60   | 1820   | 20.4   | 13.5-38.7    | 13.3-13.6     | 36.7-41.1     | 0.392         |
| ALT U/L               | 18-59 | 8996   | 22.9   | 10.0-65.6    | 9.8-10.2      | 64.2-66.6     |
|                       | ≥60   | 1820   | 18.2   | 8.8-46.7     | 8.5-9.4       | 44.2-49.1     | <0.001        |
| GGT U/L               | 18-59 | 8996   | 27.1   | 10.3-94.4    | 10.0-10.5     | 92.4-96.4     |
|                       | ≥60   | 1820   | 22.5   | 9.1-81.5     | 8.7-9.7       | 75.9-86.1     | <0.001        |
| ALP U/L               | 18-59 | 8996   | 60.1   | 37.5-95.9    | 37.2-37.9     | 95.0-96.9     |
|                       | ≥60   | 1820   | 60.5   | 38.3-99.3    | 36.5-39.3     | 97.3-102.5    | 0.113         |
| TP (g/L)              | 18-59 | 8996   | 75.0   | 67.9-81.0    | 67.8-68.2     | 80.9-81.1     |
|                       | ≥60   | 1820   | 74.1   | 66.8-82.2    | 66.5-67.2     | 81.8-82.6     | <0.001        |
| ALB (g/L)             | 18-59 | 8996   | 45.0   | 40.5-49.3    | 40.4-40.5     | 49.2-49.4     |
|                       | ≥60   | 1820   | 43.2   | 38.6-47.6    | 38.4-38.8     | 47.4-47.8     | <0.001        |
| TBIL (μmol/L)         | 18-59 | 8996   | 16.3   | 8.4-32.1     | 8.2-8.5       | 31.5-32.6     |
|                       | ≥60   | 1820   | 17.3   | 9.0-35.7     | 8.5-9.4       | 34.4-36.7     | <0.001        |
| DBIL (μmol/L)         | 18-59 | 8996   | 3.2    | 1.5-6.6      | 1.4-1.5       | 6.5-6.6       |
|                       | ≥60   | 1820   | 3.6    | 1.6-7.9      | 1.5-1.7       | 7.6-8.3       | <0.001        |
| **Female**            |       |        |        |              |               |               |
| AST U/L               | 18-59 | 11052  | 17.2   | 12.1-32.9    | 12.1-12.2     | 32.1-33.5     |
|                       | ≥60   | 1729   | 20.4   | 13.7-41.5    | 13.2-14.0     | 39.1-44.1     | <0.001        |
| ALT U/L               | 18-59 | 11052  | 13.4   | 6.7-42.9     | 6.6-6.8       | 41.3-44.4     |
|                       | ≥60   | 1729   | 16     | 8.1-47.2     | 7.7-8.4       | 44.5-50.2     | <0.001        |
| GGT U/L               | 18-59 | 11052  | 13.2   | 5.9-49.9     | 5.8-6.0       | 48.1-51.0     |
|                       | ≥60   | 1729   | 16.4   | 7.2-61.6     | 6.7-7.6       | 52.9-66.4     | <0.001        |
| ALP U/L               | 18-59 | 11052  | 52.6   | 32.9-95.3    | 32.6-33.2     | 94.4-96.4     |
Comparison of RIs between this study and other studies

The RIs established by non-parametric method are shown in Figure 4. RIs for most analytes needed to be divided according to sex, age or both. Apparently, AST, ALT, TP and ALB showed no sex partition in the whole groups. ALP and TBIL required no age partition, whereas GGT and DBIL in males and females displayed a distinct age dependency. Although differences in concentration across ages were relatively minor, 2 different age partitions were required in males for DBIL, GGT in males and DBIL in females required 3 different age partitions.

Table 2, lists the RIs obtained in this study and RIs derived from national standards[12–14]. In the meantime, studies in China[15] Asmara[16] and Canada[17] were also used to compare the differences about RIs among regions. The LL and UL of AST obtained in this study were a little higher than those of national standard, whereas reference limits of ALP and ALB were lower compared with the national standard. RIs of TP were similar to national standard.

Discussion

Evaluation of indirect method

The direct method for establishing RIs has been recommended as gold standard in recent years[18–19]. However, the indirect method based on data mining techniques and laboratory information system, which is a useful adjunct to traditional direct methods. Of note, important advantages of the indirect method include that it is faster, cheaper and easier to perform, without evaluating whether all individuals results from the database belong to the reference population. Therefore, majority of clinical laboratories prefer to choose the indirect method to establish RIs. In that respect, this study is the first study to establish RIs for serum liver function tests of the elderly in China using data from LIS.
Participants in the study

The selection of reference population is an important step to establish RIs by the indirect method. All the subjects in this study were collected from LIS in the hospital. In order to improve the reliability of the data, appropriate screening criteria had been set to obtain healthy individuals. For example, since the data were collected from LIS within one year, participants who had undergone duplicate tests were excluded, for they were at a potential risk of disease.

Comparison of RIs between this study and other studies

The main findings included the following facts that RIs for serum TP and ALB didn't require sex partition, which was consistent with national standard and the multi-center study[13, 15]. The downward tendency in serum TP levels may be due to the decrease in the volume and number of liver cells with age, which was consistent with the study in Canada[17]. Similarly, abnormal liver function, reduced protein intake and poor absorption are more likely to occur in the elderly, resulting in decreased TP synthesis ability[20]. And elderly individuals have weaker physical quality, high risk of fracture and are more susceptible to infections, which increase the consumption of a large number of serum proteins[21].

The data also demonstrated same results as those of Asmara[16], namely that AST and ALT did not reveal a distinct sex dependency in the elderly. The results were also in agreement with an already established knowledge that ALT levels were not affected by sex or metabolic factors[22]. However, the RIs were slightly different. The UL of AST was similar compared with study in Canada, whereas its LL was lower. Both UL and LL of ALT were lower than those in Canada, the reasons may be associated with differences in race, laboratory equipment, life style and eating habit.

Sex and age partitions were required for the remaining four analytes. GGT in males was higher than that in females, which was consistent with other studies[15, 17, 18]. GGT is not only an index of liver dysfunction, but also a marker of alcohol intake[23]. Since the proportion of drinking in males is higher than that in females, which may be the reason for higher values in males. ALP is closely mirrored bone growth. The concentration was at a relatively stable level in the elderly[24]. The reason may be that the development of bone growth in the elderly has reached a stable stage and without large fluctuation. ALP levels were higher in females than that in males, same as those results that displayed in other studies[25]. Some studies also demonstrated that changes of postmenopausal hormone level can make bone metabolism reach to a high transition state, which can increase bone formation and bone resorption[26]. This may be the main reason for higher RI in females. But the UL and LL of ALP were different from those studies. It was inferred that this difference may be caused by race, climate and geography. Similarly, the method for selecting reference individuals may be another contributing factor. In this study, concentrations of TBIL were higher when compared to other studies. However, they were comparable to that from Jiangsu province[27]. The proportion of OOR values for TBIL is 20.6%. This means 20.6% of enrolled study participants would be regarded as abnormal results, and the one who does not need treatment would end up being treated due to the use of inappropriate reference values. Concentrations of DBIL increased with age in males and females in this study, which showed the same results as that in
Asmara. Of note, compared with other studies, different age groups were required for DBIL in this study. It may be due to the differences in the number of reference population among age subgroups and statistical methods.

It is worth noting that although the sex and age differences of some parameters in our study reveal a statistically significant difference, the differences of these values were minor. The reason could be that these differences do not show enough clinical significance to ensure the establishment of different standards between males and females or different age subgroups. Therefore, accumulating more data from other sources will be more helpful to establish reasonable RIs.

Limitations

Unfortunately, this study still has some limitations. We aimed to establish RIs for the elderly, so some individuals with underlying diseases or subclinical conditions may be included in the study. Data were distributed unevenly among age subgroups, so data for participants aged 80 or older should be increased. Despite the limitations, RIs obtained in this study should provide valuable guidelines and make the interpretation of clinical data in elderly more reasonable.

Conclusion

In summary, the RIs of liver function in the elderly are different from those of adults. In this study, RIs are established according to sex and age, which provide a reference of biochemical test items for the elderly. Establishing RIs for elderly is, however, complicated as it remains unclear if changes in levels of analytes are affected by aging or disease. Therefore, RIs should be re-accessed periodically to ensure that these reference values are still suitable for the population of nowadays.

Abbreviations

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: Gamma-glutamyltransferase; ALP: Alkaline phosphatase; TP: Total protein; Alb: Albumin; TBIL: Total bilirubin; DBIL: Direct bilirubin; LL: Lower limits; UL: Upper limits; RI: Reference interval; LIS: Laboratory information system

Declarations

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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**Authors’ contributions**

SZY gathered and analysed the data, and wrote the first draft of the manuscript. CJT was committed to statistical processing. ZQ and XJC conceived the study and revised the article. All authors have reviewed and edited the manuscript and approved the final version of the manuscript.

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**Ethics approval and consent to participate**

This study was approved by the Ethics Committee of the First Hospital of Jilin University. (NO:2021-543). This was a retrospective observational study, all data was obtained from laboratory information system and only medical records were analyzed. The need for informed consent (both written and oral) was waived by the Committee. This waiver does not adversely affect the rights and welfare of the subjects. All procedures involving human participants were performed in accordance with the ethical standards of the Ethics Committee for Clinical Research of the First Hospital of Jilin University and with the Declaration of Helsinki and its later amendments.

**Competing interests**

The authors declare that they have no competing interests.

**Consent for publication**

Not applicable.

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Table

Due to technical limitations, table 2 is only available as a download in the Supplemental Files section.

Figures
Figure 1

The selection criteria for liver function tests.
Figure 2

Sex-specific distributions of liver function tests in the elderly. The distance between the top and the bottom horizontal lines represents the range of reference values. The dots represent male (·) and the asterisks represent female (*), respectively. AST = aspartate aminotransferase; ALT = alanine aminotransferase; GGT = gamma-glutamyltransferase; ALP = alkaline phosphatase; TP = total protein; ALB = albumin; TBIL = total bilirubin; DBIL = direct bilirubin.
Figure 3

Sex- and age-specific continuous percentile curves of all analytes. The sequences of each pair of curves from top to down are expressed as P97.5, P50 and P2.5 for males and females. AST = aspartate aminotransferase; ALT = alanine aminotransferase; GGT = gamma-glutamyltransferase; ALP = alkaline phosphatase; TP = total protein; ALB = albumin; TBIL = total bilirubin; DBIL = direct bilirubin
| Test      | M | F | M+F |
|-----------|---|---|-----|
| AST, U/L  | 13.5-40.3 | M+F |
| ALT, U/L  | 8.7-48.5 | 8.2-42.7 | 6.4-42.0 | M+F |
| GGT, U/L  | 9.1-82.1 | 8.5-84.6 | 10.5-74.6 | M+F |
| ALP, U/L  | 38.3-99.3 | 41.7-115.8 | M+F |
| TP, g/L   | 67.1-82.4 | 66.4-82.3 | M+F |
| ALB, g/L  | 39.0-47.5 | 38.0-46.7 | 37.5-45.7 | M+F |
| TBIL, µmol/L | ≤31.6 | ≤23.6 | M+F |
| DBIL, µmol/L | ≤6.9 | ≤4.8 | ≤8 | M+F |

**Figure 4**

Sex- and age-specific RIs of liver function tests in the healthy elderly population M: male; F: female. AST = aspartate aminotransferase; ALT = alanine aminotransferase; GGT = gamma-glutamyltransferase; ALP = alkaline phosphatase; TP = total protein; ALB = albumin; TBIL = total bilirubin; DBIL = direct bilirubin

**Supplementary Files**

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- Table2.xls