SCINDEKS
Serbian Citation Index
J Med Biochem 2023; 42 (4) DOI: 10.5937/jomb0-40154

**UDK 577.1 : 61** **ISSN 1452-8258**

*J Med Biochem 42: 630–637, 2023*

*Original paper*
*Originalni naučni rad*

# RETROSPECTIVE STUDY TO IDENTIFY HOMOCYSTEINE REFERENCE INTERVALS IN HEALTHY CHINESE 60 YEARS OF AGE AND ABOVE

## RETROSPEKTIVNA STUDIJA ZA IDENTIFIKACIJU REFERENTNIH INTERVALA HOMOCISTEINA KOD ZDRAVIH KINEZA STAROSTI 60 GODINA I VIŠE

*Jianmin Zong[1], Yue Sun[2]*

*[1]Department of Laboratory Medicine, Nanjing Prevention and Treatment Center for Occupational Diseases, Nanjing, Jiangsu, China*
*[2]Department of Laboratory Medicine, Lianyungang Hospital Affiliated to Nanjing University of Chinese Medicine, Lianyungang, Jiangsu, China*

## Summary

**Background:** Homocysteine (Hcy) are associated with many age-related diseases. Heterogeneous physiology with aging combined with unresolved assays standardization necessitates the establishment of specific Hcy reference intervals (RIs) applicable to the elderly. This retrospective study aimed to identify Hcy RIs in the elderly aged 60 years and older from a hospital in Jiangsu Province, China.
**Methods:** Data from individuals undergoing routine physical examinations were collected. Hcy were measured on Hitachi 7600 analyzer using hydrolase-based enzymatic cycling method. Outliers were identified by Dixon methods. Age- and gender-specific differences were estimated by nonparametric tests. Factors affected Hcy were assessed using multivariate linear regression. RIs with 90% confidence intervals were determined by nonparametric method.
**Results:** A total of 2594 individuals were included. Hcy levels increased with age ($r=0.248$, $p<0.001$). Males have consistently higher Hcy levels (median (interquartile range): 11.95 (8.89–15.30) mmol/L) than females (9.65 (7.05–12.69) mmol/L; $p<0.001$). Multivariate adjustment analysis showed correlations between Hcy and gender ($\beta=0.188$, $p<0.001$), age ($\beta=0.427$, $p<0.001$) were significant. The Hcy RIs were 5.10–25.46 mmol/L for males, and 4.14–18.91 mmol/L for females, respectively.
**Conclusions:** This study identified age- and gender-specific Hcy RIs in the elderly, which may guide clinicians in interpreting laboratory findings and clinical management.

**Keywords:** homocysteine, reference intervals, indirect methods, aged

## Kratak sadržaj

**Uvod:** Homocistein (Hcy) je povezan sa mnogim bolestima vezanim za uzrast. Heterogena fiziologija sa starenjem u kombinaciji sa nerazjašnjenom standardizacijom testova zahteva uspostavljanje specifičnih Hcy referentnih intervala (RI) primenljivih na starije osobe. Ova retrospektivna studija imala je za cilj da identifikuje Hcy RI starosti 60 godina i više iz bolnice u provinciji Jiangsu, Kina.
**Metode:** Prikupljeni su podaci od pojedinaca koji su bili podvrgnuti rutinskim fizičkim pregledima. Hcy je izmeren na Hitachi 7600 analizatoru korišćenjem metode enzimskog ciklusa zasnovanog na hidrolazi. Izuzetne vrednosti su identifikovane Diksonovim metodama. Razlike po uzrastu i polu su procenjene neparametarskim testovima. Faktori koji utiču na Hcy su procenjeni korišćenjem multivarijantne linearne regresije. RI sa 90% intervala poverenja određeni su neparametrijskom metodom.
**Rezultati**: Uključeno je ukupno 2594 osobe. Nivo Hcy se povećavao sa godinama ($r=0,248$, $p<0,001$). Muškarci imaju konstantno više Hcy nivoe (medijan (interkvartilni opseg): 11,95 (8,89–15,30) μmol/L) od žena (9,65 (7,05–12,69) μmol/L; $p<0,001$). Multivarijantna analiza prilagođavanja pokazala je da su korelacije između Hcy i pola ($\beta=0,188$, $p<0,001$), i starosti ($\beta=0,427$, $p<0,001$) bile značajne. Hcy RI su bili 5,10–25,46 μmol/L za muškarce, odnosno 4,14–18,91 μmol/L za žene.
**Zaključak:** Ova studija je identifikovala Hcy RI specifične za uzrast i pol kod starijih osoba, što može poslužiti kliničarima u tumačenju laboratorijskih nalaza i kliničkom upravljanju.

**Ključne reči:** homocistein, referentne vrednosti, indirektne metode, starenje

*Address for correspondence:*

Jianmin Zong
Department of Laboratory Medicine, Nanjing Prevention and Treatment Center for Occupational Diseases, No.4 Hua Yuan Road, Nanjing, Jiangsu, China, 210042.
e-mail: zong_jm@163.com

## Introduction

Homocysteine (Hcy) has been reported to be associated with many age-related diseases such as cardio-cerebrovascular diseases, neurodegenerative diseases, cognitive impairment, osteoporotic fractures, and even cancer (1–3).

China is aging, data from the National Census in 2021 released that people aged 60 and above have reached 264 million, accounting for 18.7% of the total population (4). Hcy has gaining importance in geriatric healthcare delivery and an appropriate reference interval (RI) is of practical significance to be established.

There is still a gap between Hcy RI establishment and application. To date, standardization of assays remains unresolved due to the lack of certified reference method and material (5), multiple assays such as chromatography, enzymatic assay and immunoassay are used in different laboratories and the variations among results are actually considerable (6). Regardless of analytical platforms, intervals of 5–15 mmol/L (two-sided) or <15 mmol/L (right-sided) are widely adopted as Hcy RIs in practice (7, 8), which seems to be clinically inappropriate.

Heterogeneous changes accompanied by normal aging result in much wider intervals in the elderly than in adults and they are not sufficient to be considered a pathological condition, which has been well characterized by previous studies. Since factors such as age, gender, and ethnicity are known to affect Hcy results (9), we suspect the non-partitioned Hcy RI might significantly complicate the interpretation of laboratory findings in the elderly.

Although clinical laboratories have been recommended for decades to establish RIs appropriate to the assay method and local population (10), more than 80% of laboratories in China prefer to adopt RI provided by manufacturer (11), limited studies on RIs establishment are available so far, and the subjects are mainly constricted to adult (12–16), elderly has not been a detailed description yet due to the limited sample size.

Due to difficulties of recruiting enough elderly healthy individuals, indirect methods were proposed in the Clinical and Laboratory Standards Institute (CLSI) EP28-A3c guideline (10). In this approach, data were extracted from the database by applying exclusion criteria and statistical methods, rather than collecting samples from recruited subjects, which is relatively feasible for individual laboratories.

Therefore, this retrospective study aimed to analyze the effects of indicators such as gender and age on Hcy and determine the RIs in the elderly aged 60 years and older by indirect method.

## Materials and Methods

*Study design*

To extract data from relatively healthy subjects, we retrieved data from individuals undergoing routine physical examinations for periodic health screening rather than all outpatients from January 1, 2018 to June 30, 2021. The last available data of the same individual was retained. The following exclusion criteria were applied as extra precautions to remove underlying pathological results may be present in the database. Individuals with missing records will be considered unqualified and their test results will be excluded.

1. Self-reported history or drugs treatment in past history records, including endocrine diseases, autoimmune disease, cardiovascular diseases, respiratory diseases, hematological diseases, cancer.

2. Abnormalities found in ultrasound, electrocardiogram and X-ray examination.

3. Body mass index (BMI) ≥ 28 kg/m$^2$ or 18.5 kg/m$^2$.

4. Hypertension: systolic blood pressure (SBP) ≥140 mmHg or diastolic blood pressure (DBP) ≥90 mmHg.

5. Hyperlipidemia: total cholesterol (TC) ≥6.22 mmol/L, triglyceride (TG) ≥1.70 mmol/L.

6. Hyperglycemia: fasting blood glucose (FBG) ≥ 7.0 mmol/L.

7. Hemoglobin (HGB)<120 g/L (for males) or 110 g/L (for females); white blood cell (WBC) >13.0×10$^9$/L or<3.0×10$^9$/L; platelet (PLT) >350×10$^9$/L or<100×10$^9$/L; high sensitivity C-reaction protein (hsCRP) >10.0 mg/L.

8. Abnormal liver and kidney function: albumin (ALB) <40 g/L; alanine aminotransferase (ALT) >50 U/L (for males) or >40 U/L (for females); creatinine (Crea) >111 mmol/L (for males) or>81 mmol/L (for females); urea>9.5 mmol/L (for males) or>8.8 mmol/L (for females).

9. Positive results of hepatitis B surface antigen (HBsAg), anti-hepatitis C virus (anti-HCV), or anti-human immunodeficiency virus (anti-HIV).

The Ethics Committee of Lianyungang Hospital affiliated to Nanjing University of Traditional Chinese Medicine deemed the study exempt from review. This retrospective study was conducted on anonymization-based already available data and informed consent was waived.

*Measurements*

About 5 mL of venous blood was drawn into gel separator tubes (BD biosciences, New Jersey, USA) after an overnight fast. All samples were collected

between 7:00 am and 11:00 am. Samples were centrifuged for 10 minutes at 1,500 g within 1 hour after collection. Lipemic or hemolyzed samples were considered ineligible. Samples were loaded into Hitachi 7600 automatic analyzer (Hitachi Co., Ltd, Tokyo, Japan) within 2 hours, Serum Hcy concentrations were analyzed by hydrolase-based enzymatic cycling method with reagents provided by Beijian Xinchuangyuan (Beijian BJ Biotech Co., Ltd, Beijing, China). The linearity range is 3.0–45.0 mmol/L. Samples will be diluted with normal saline and retested when the detected value of the sample exceeds the linear range. The inter-assay coefficient of variations was 7.85%. Operations were performed according to the standard operating procedures. Internal quality controls were performed prior to every analytical run and deemed to be valid. We also participated in the external quality assessments of the National Center for Clinical Laboratories twice a year to ensure the results were credible.

*Statistics*

Statistical analyses were performed on SPSS 19.0 (SPSS Inc., Chicago, USA) and GraphPad PRISM 9.0 for Windows (GraphPad Software, Inc., San Diego, CA, USA). Outliers were identified using Dixon method. Normality of data was tested by Kolmogorov-Smirnov test. Gaussian distributed data were expressed as means and standard deviation, whereas non-Gaussian distributed data were expressed as medians and interquartile range. Spearman's test was performed to assess the correlation between age and Hcy values. Age- and gender-specific differences were estimated by Mann-Whitney U test and Kruskal-Wallis H test as appropriate. Multivariate linear regression was used to evaluate the effects of sex, age and other analytes on Hcy levels. RIs were determined by a non-parametric method which expressed as $2.5^{th}$ and $97.5^{th}$ percentiles with 90% confidence intervals calculated by a bootstrap-resampling procedure. Two-tailed p value$<$0.05 was considered statistically significant.

## Results

*Baseline characteristics of subjects*

2594 individuals of 60–97 years of age, including 1606 males (61.9%) and 988 females (38.1%) were collected. All of them were Han ethnicity. The Mann-Whitney U test showed gender differences with higher blood pressure, WBC, HGB, ALB, ALT, Crea, TG, FBG levels in males and higher PLT in females (*Table I*). Data showed a non-Gaussian distribution according to Kolmogorov-Smirnov test, thus nonparametric methods were used.

**Table I** Baseline characteristics of included individuals.

| Index | Total (n=2594) | Males (n=1606) | Females (n=988) | p value[a] |
|---|---|---|---|---|
| Age (years) | 64(64, 75) | 69(64, 76) | 67(63, 73) | <0.001 |
| SBP (mmHg) | 116(108,126) | 120(112,128) | 111(103,120) | <0.001 |
| DBP (mmHg) | 72(65,78) | 75(69,81) | 66(61,73) | <0.001 |
| WBC ($\times10^9$/L) | 6.02(5.02,7.18) | 6.09(5.18,7.20) | 5.93(4.73,7.12) | <0.001 |
| HGB ($\times10^9$/L) | 142(133,152) | 148(140,157) | 134(127,140) | <0.001 |
| PLT ($\times10^9$/L) | 190(159,226) | 180(154,214) | 207(173,244) | <0.001 |
| hsCRP (mg/L) | 1.00(0.93,2.72) | 1.00(0.93,2.91) | 1.00(0.90,2.58) | 0.286 |
| ALB (g/L) | 47.6(46.1,49.2) | 47.9(46.4,49.4) | 47.3(45.7,48.7) | <0.001 |
| ALT (U/L) | 18(13,24) | 19(15,26) | 16(12,21) | <0.001 |
| Crea (μmol/L) | 67(55,78) | 75(68,83) | 53(48,59) | <0.001 |
| Urea (mmol/L) | 4.98(4.21,5.88) | 5.27(4.56,6.15) | 4.46(3.75,5.29) | <0.001 |
| TC (mmol/L) | 4.78(4.19,5.31) | 4.77(4.18,5.31) | 4.81(4.21,5.34) | 0.057 |
| TG (mmol/L) | 1.24(0.85,1.64) | 1.26(0.89,1.63) | 1.21(0.79,1.65) | <0.001 |
| FBG (mmol/L) | 5.33(5.05,5.65) | 5.38(5.09,5.71) | 5.26(5.02,5.56) | <0.001 |

[a]Tested with Mann-Whitney U test for variables between genders.
Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; WBC, white blood cell; HGB, hemoglobin; PLT, platelet; hsCRP, high sensitivity C-reaction protein; ALB, albumin; ALT, alanine aminotransferase; Crea, creatinine; TC, total cholesterol; TG, triglycerides; FBG, fasting blood glucose.

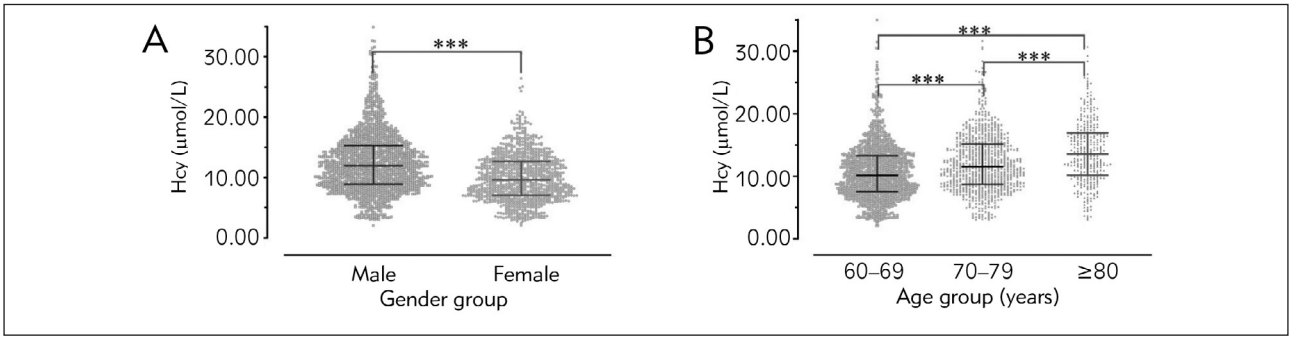


**Figure 1** Distribution of Hcy stratified by (A) gender and (B) age.
The lines and error bars represent medians and interquartile ranges, respectively. ***P<0.001

**Table II** Multivariate linear regression of the association between various factors with Hcy.

| Factors | Unadjusted | | | Age- and sex-adjusted | | |
|---|---|---|---|---|---|---|
| | B (SE) | Beta | p value | B (SE) | Beta | p value |
| Gender (0: female 1: male) | 2.477 (0.194) | 0.243 | <0.001 | 1.915 (0.176) | 0.188 | <0.001 |
| Age (years) | 0.307 (0.012) | 0.452 | <0.001 | 0.291 (0.012) | 0.427 | <0.001 |
| SBP (mmHg) | 0.061 (0.008) | 0.147 | <0.001 | 0.017 (0.008) | 0.041 | 0.026 |
| DBP (mmHg) | 0.083 (0.011) | 0.150 | <0.001 | 0.010 (0.010) | 0.018 | 0.343 |
| WBC ($\times10^9$/L) | 0.103 (0.060) | 0.034 | 0.086 | -0.017 (0.053) | -0.005 | 0.751 |
| HGB ($\times10^9$/L) | 0.001(0.007) | 0.003 | 0.867 | -0.015(0.007) | -0.041 | 0.404 |
| PLT($\times10^9$/L) | -0.009 (0.002) | -0.088 | <0.001 | 0.000[a] (0.002) | 0.000[a] | 0.998 |
| ALB (g/L) | 0.162 (0.041) | 0.077 | <0.001 | 0.097 (0.036) | 0.046 | 0.048 |
| ALT (U/L) | 0.051 (0.012) | 0.086 | <0.001 | 0.022 (0.010) | 0.037 | 0.056 |
| Crea (μmol/L) | 0.061 (0.006) | 0.190 | <0.001 | -0.007 (0.008) | -0.022 | 0.370 |
| Urea (mmol/L) | 0.322 (0.076) | 0.083 | <0.001 | -0.095 (0.070) | -0.024 | 0.179 |
| TC (mmol/L) | 0.175 (0.121) | 0.029 | 0.146 | 0.135 (0.105) | 0.056 | 0.040 |
| TG (mmol/L) | 0.137 (0.238) | 0.011 | 0.564 | -0.003 (0.208) | 0.000[a] | 0.989 |
| FBG (mmol/L) | 0.752(0.192) | 0.077 | <0.001 | 0.199(0.169) | 0.020 | 0.239 |

Unstandardized coefficients were expressed as B (B-coefficient) and SE (standard error); standardized coefficients were expressed as Beta.
[a]Coefficients are calculated to three decimal figures.
Abbreviations: Hcy, homocysteine; SBP, systolic blood pressure; DBP, diastolic blood pressure; WBC, white blood cell; HGB, hemoglobin; PLT, platelet; ALB, albumin; ALT, alanine aminotransferase; Crea, creatinine; TC, total cholesterol; TG, triglycerides; FBG, fasting blood glucose.

**Table III** Gender- and age-specific Hcy reference intervals (μmol/L).

| Age, years | Male Reference intervals | | | | Female Reference intervals | | | |
|---|---|---|---|---|---|---|---|---|
| | Sample, n | Median | LL (90%CI) | UL (90%CI) | Sample, n | Median | LL (90%CI) | UL (90%CI) |
| 60–69 | 836 | 11.11 | 4.66 (4.01–5.06) | 23.37 (22.66–25.72) | 613 | 8.86 | 4.10 (4.06–4.16) | 16.55 (15.45–16.96) |
| 70–79 | 523 | 12.26 | 5.42 (5.08–5.69) | 26.69 (24.05–31.30) | 283 | 10.28 | 4.51 (4.47–5.02) | 20.16 (18.99–24.86) |
| ≥80 | 247 | 14.04 | 6.09 (5.90–6.35) | 25.77 (24.60–29.10) | 92 | 12.00 | 5.47 (5.12–6.02) | 19.66 (18.91–20.97) |
| ≥60 | 1606 | 11.95 | 5.10 (4.87–5.28) | 25.46 (24.00–26.69) | 988 | 9.65 | 4.14 (4.09–4.30) | 18.91 (18.02–19.36) |

Abbreviations: Hcy, homocysteine; LL, lower limit; UL, upper limit; CI, confidence interval.

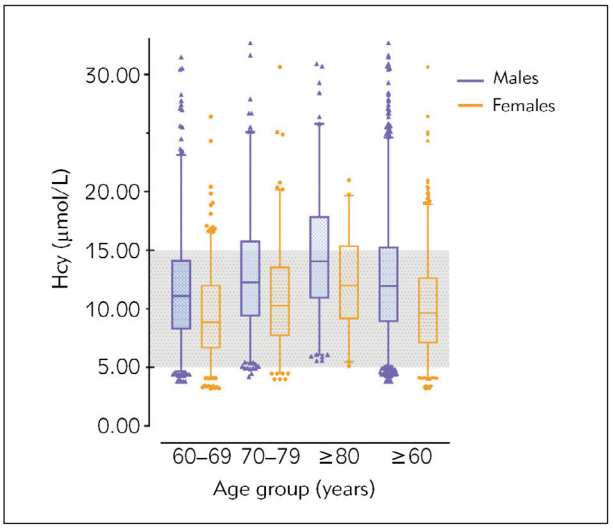


**Figure 2** Hcy reference intervals in males and females 60 years of age and above.

The boxplot describes the medians (lines in the boxes), 25th–75th percentiles (limits of boxes), and 2.5th–97.5th percentiles (vertical lines) of Hcy concentrations. Grey area represents the currently recognized reference interval (5–15 μmol/L).

*Distribution of Hcy levels according to gender and age*

Males had consistently higher Hcy levels (11.95 (8.89–15.30) mmol/L) than females (9.65 (7.05–12.69) mmol/L; $p<0.001$). Hcy levels showed steady upward movement in the three randomly defined age groups (60–69, 70–79 and ≥80 years) and significant differences were noted in pairwise comparisons ($p<0.001$) (*Figure 1*).

*Effects of confounders on Hcy levels*

The multivariate liner regression analysis revealed that Hcy was positively associated with many variables including gender, age, blood pressure, PLT, ALB, ALT, Crea, Urea, TC, TG and FBG ($p<0.05$). After age- and sex-adjustment, gender ($\beta=0.188$, $p<0.001$), age ($\beta=0.427$, $p<0.001$), SBP ($\beta=0.041$, $p=0.026$), ALB ($\beta=0.046$, $p=0.048$) and TC ($\beta=0.056$, $p=0.040$) remained statistically significant (*Table II*).

*Established Hcy RIs in the elderly*

Based on the results above, we resulted in gender- and age-specific RIs in the elderly (*Table III*). And

**Table IV** Hcy reference intervals determined in previous studies (μmol/L).

| Author | Year | Source | Assay | Age, years | Sample size, n | Reference Interval |
|---|---|---|---|---|---|---|
| Si et al. (17) | 2022 | Chinese | Enzymatic assay | 61–90 | M:8570<br>F: 2886 | 14.70 (10.00–20.90)<br>12.40 (8.40–16.30) |
| Cui et al. (12) | 2022 | Chinese | LC-MS/MS | 21–79 | M:710<br>F: 843 | 10.66 (6.09–17.00)<br>8.19 (4.61–14.61) |
| Adeli et al. (13) | 2015 | Canadian | Enzymatic/RS | 26–79 | M:680<br>F: 420 | 8.30 (5.20–14.10)<br>6.90 (3.70–10.90) |
| Lahiri et al. (14) | 2014 | Indian | Enzymatic assay | 20–81 | M:636<br>F: 652 | 11.44 (6.50–16.38) |
| Kweon et al. (18) | 2014 | South Korean | FPIA | 65–74 | M:414<br>F: 630 | 7.93 (5.12–13.90)[a]<br>6.47 (3.95–10.65)[a] |
| Moon et al. (15) | 2011 | South Korean Hcy | FPIA | 21–65 | M:220<br>F: 160 | 11.59 (7.26–19.21)[b]<br>8.50 (5.50–14.99)[b] |
| Taskin et al. (16) | 2006 | Turk | HPLC | 22–81 | M:118<br>F: 41 | 9.52 (5.57–20.55)[b]<br>7.39 (4.40–16.20)[b] |
| Ganji et al. (19) | 2006 | American (non-Hispanic white)<br>American (non-Hispanic black)<br>American (Hispanic) | FPIA<br>FPIA<br>FPIA | >70<br>>70<br>>70 | M:510<br>F: 515<br>M:84<br>F: 114<br>M:142<br>F: 143 | 10.80 (7.28–19.52)[a]<br>9.53(6.15–19.05)[a]<br>11.16(7.02–21.74)[a]<br>10.73 (6.67–26.19)[a]<br>11.19 (7.19–19.12)[a]<br>10.63 (6.39–17.71)[a] |
| Molero et al. (20) | 2006 | Venezuelan (Hispanic) | FPIA | >55 | M:601<br>F: 302 | 12.60 (7.43–23.52)<br>11.00 (6.02–20.46) |

Values are presented as the medians (2.5th–97.5th percentiles) unless otherwise indicated.
[a] Values are presented as the medians (5th–95th percentiles); [b] Values are presented as the means (2.5th–97.5th percentiles)
Abbreviations: Hcy, homocysteine; M, male; F, female; LC-MS/MS: liquid chromatography-tandem mass spectrometry; RS, reflectance spectrophotometry; FPIA, fluorescence polarization immunoassay; HPLC, high performance liquid chromatography.

due to the overlap of 90% confidence intervals of the upper and lower limit, RIs for males and females combining the different age groups were calculated as well. Gender- and age-partitioned Hcy RIs compared to those of manufactures are shown in *Figure 2*.

*Comparison of Hcy RIs across previous studies*

The Hcy RIs reported across studies showed considerable differences (*Table IV*). Most studies resulted in gender- partitioned RIs; some of them also provided RIs by different age subgroups.

## Discussion

In the present study, we extracted data from the database from a hospital in Jiangsu Province, China by applying exclusion criteria and statistical methods and identified Hcy RIs in 2594 healthy individuals 60 years of age and above. Higher than the current RI (5–15 mmol/L), RIs established this study were 5.10–25.46 mmol/L for males, and 4.14–18.91 mmol/L for females, respectively.

The findings proved elderly yield higher Hcy values as well as wider ranges than adults. Decreased renal function, losses of muscle mass and reduced physical activity in the elderly are reported as determinants (21, 22). Absorption dysfunction accompanied by aging also put the elderly at higher risk of folate and vitamin B12 deficiencies. Males have higher Hcy levels than females. Although gender differences have been reported by many studies, findings are inconsistent in the elderly. Fonseca et al. (23) found that differences diminish after menopause. No significant association of estradiol and Hcy levels in elderly men was observed by Nakhai et al. (24). However, our finding of significantly lower Hcy levels in postmenopausal women compared to men were consistent with Cohen et al. (25), suggesting the differences between gender with regard to Hcy were only partially explained by the Hcy-lowering effect of estrogen (26), more determinants need to be taken into account. Gender differences exist in folate and vitamin B12 levels. A NHANES survey indicated that elderly males are more likely to be deficient in these vitamins than females (27). A prospective study in healthy Swiss seniors also found significantly lower levels of these vitamins in males (28). Males tend to produce more creatine due to more muscle mass, which connected with methyl transfer, leading to Hcy formation (29). Additionally, females are less possible exposed to smoke and alcohol consumption, which could influence lowering Hcy levels (30).

Geographical and ethnic differences are significant. Hcy levels were approximately 13 mmol/L in China (17) whereas in some countries the values were even less than 10 mmol/L (13, 16, 18). Chinese tend to yield higher Hcy concentrations, which could be ascribed to the gene-environment interaction of a higher prevalence of methylenetetrahydrofolate reductase C677T (MTHFR C677T) mutation (31) and a dietary with no or partial folic acid fortification (32).

Moreover, results could be more comparable despite bias with those of other studies based on Chinese or other closely related ethnicities. In Si et al.'s study (17), Hcy RIs in 60–90 years were 10.00–20.90 mmol/L for males and 8.40–16.30 mmol/L for females respectively. A Korean study showed that the upper limits of RIs for male and female were 19.21 mmol/L and 14.99 mmol/L (15). The right-sided RIs determined by Ma et al. (33) were 15.80 mmol/L for males and 13.60 mmol/L for females, respectively. These lower values may be due to the fact that their subjects included people aged 18–60 years old, and the latter also determined upper limits from the 95th percentiles rather than the 97.5th percentiles.

Except age and gender, the multivariate-adjusted analysis showed SBP, ALB and TC was also correlated to Hcy concentrations. Despite the statistical significance, the slopes of regression equations are so small that small changes in Hcy require larger changes in these variables. It is worth mentioning, however, that the correlation between blood pressure and Hcy was not as strong as expected, which we speculate is due to the relatively lower blood pressure of the subjects, thus weakening the significance. Data in this study were extracted in normotensive subjects after exclusion of abnormal results. Additionally, the effect of large arterial stiffness in the elderly could result in downward movement of blood pressure to some extent (34). It also might be related to the fact that people undergoing physical examinations were required to go through an overnight fast and have their blood pressures measured at rest.

There are also some limitations that need to be mentioned. Data in this study were extracted from the database rather than volunteer recruitment. Information including smoke, alcohol consumption and vitamin supplementation were not available in the database. Given the limitations of approaches, the findings are supposed to be informative rough estimates. Besides, standardization of Hcy assay remains unresolved, variation exists among platforms and reagents. RIs based on enzymatic assays cannot be expanded to other methods.

## Conclusion

The findings from this study identified age- and gender-related Hcy RIs in the healthy population 60 years of age and above in Jiangsu Province, China, which may provide personalized tools in interpreting laboratory findings and clinical management of this patient population.

## Conflict of interest statement

All the authors declare that they have no conflict of interest in this work.

## References


1. Djuric D, Jakovljevic V, Zivkovic V, Srejovic I. Homocysteine and homocysteine-related compounds: an overview of the roles in the pathology of the cardiovascular and nervous systems. Can J Physiol Pharmacol 2018; 96: 991–1003.
2. Kuo HK, Sorond FA, Chen JH, Hashmi A, Milberg WP, Lipsitz LA. The role of homocysteine in multisystem age-related problems: a systematic review. J Gerontol A Biol Sci Med Sci 2005; 60: 1190–201.
3. Ostrakhovitch EA, Tabibzadeh S. Homocysteine and age-associated disorders. Ageing Res Rev 2019; 49: 144–64.
4. National Bureau of Statistics of China. Communiqué of the Seventh National Population Census (No.5); 2021. Available from: http: //www.stats.gov.cn/english/PressRelease/202105/t20210510_1817190.html.
5. Ubbink JB. Assay methods for the measurement of total homocyst(e)ine in plasma. Semin Thromb Hemost 2000; 26: 233–41.
6. Ducros V, Demuth K, Sauvant MP, Quillard M, Caussé E, Candito M, et al. Methods for homocysteine analysis and biological relevance of the results. J Chromatogr B Analyt Technol Biomed Life Sci 2002; 781: 207–26.
7. Marković-Boras M, Čaušević A, Ćurlin M. A relation of serum homocysteine and uric acid in Bosnian diabetic patients with acute myocardial infarction. J Med Biochem 2021; 40(3): 261–69.
8. Kang SS, Wong PWK, Malinow MR. Hyperhomocyst(e)inemia as a risk factor for occlusive vascular disease. Annu Rev Nutr 1992; 12: 279–98.
9. Refsum H, Smith AD, Ueland PM, Nexo E, Clarke R, Mcpartlin J, et al. Facts and recommendations about total homocysteine determinations: an expert opinion. Clin Chem 2004; 50: 3–32.
10. CLSI. Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline-Third Edition. Wanye, PA; Clinical and Laboratory Standards Institute; 2010.
11. Zhang L, Wang W, Zhong K, He F, Wang Z. Survey and analysis on sources of reference intervals and distribution of medical decision levels in NT-proBNP, BNP and homocysteine. Chinese Journal of Cardiology 2015; 43: 965–68.
12. Cui X, Xu J, Liu M, Ren G, Cao Y. Establishment of reference intervals of homocysteine, cysteine, and methionine in apparently healthy Chinese adults. Scand J Clin Lab Invest 2022; 82: 232–37.
13. Adeli K, Higgins V, Nieuwesteeg M, Raizman JE, Chen Y, Wong SL, et al. Complex reference values for endocrine and special chemistry biomarkers across pediatric, adult, and geriatric ages: establishment of robust pediatric and adult reference intervals on the basis of the Canadian Health Measures Survey. Clin Chem 2015; 61: 1063–74.
14. Lahiri KD, Datta H, Das HN. Reference interval determination of total plasma homocysteine in an Indian population. Indian J Clin Biochem 2014; 29: 74–8.
15. Moon HW, Whang DH, Ko YJ,Joo SY, Yun YM, Hur M, et al. Reference interval and determinants of the serum homocysteine level in a Korean population. J Clin Lab Anal 2011; 25: 317–23.
16. Taskin G, Yilmaz SE, Yildirimkaya M, Nadirler F, Halloran M, Ayoglu FN, et al. Plasma total homocysteine levels in a healthy Turkish population sample. Acta cardiologica 2006; 61: 35–42.
17. Si TW, Zhang WQ, Xia F, Wang YP, Liu DQ,Wu QW. Reference intervals of homocysteine in apparently healthy Chinese Han ethnic adults. J Lab Med 2022; 46: 125–32.
18. Kweon SS, Lee YH, Jeong SK, Nam HS, Park KS, Choi SW, et al. Methylenetetrahydrofolate reductase 677 genotype-specific reference values for plasma homocysteine and serum folate concentrations in korean population aged 45 to 74 years: the Namwon study. J Korean Med Sci 2014; 29: 743–7.
19. Ganji V, Kafai MR. Population reference values for plasma total homocysteine concentrations in US adults after the fortification of cereals with folic acid. The American journal of clinical nutrition 2006; 84: 989–94.
20. Molero AE, Altimari CC, Duran DA, Garcia E, Pino RG, Maestre GE. Total plasma homocysteine values among elderly subjects: findings from the Maracaibo Aging Study. Clin Biochem 2006; 39, 1007–15.
21. Gale CR, Ashurst H, Phillips NJ, Moat SJ, Bonham JR, Martyn CN. Renal function, plasma homocysteine and carotid atherosclerosis in elderly people. Atherosclerosis 2001; 154: 141–46.
22. Alomari MA, Khabour OF, Gharaibeh MY, Qhatan RA. Effect of physical activity on levels of homocysteine, folate, and vitamin B12 in the elderly. Phys Sportsmed 2016; 44: 68–73.
23. Fonseca V, Guba SC, Fink LM. Hyperhomocysteinemia and the endocrine system: implications for atherosclerosis and thrombosis. Endocr Rev 1999; 20: 738–59.
24. Nakhai Pour HR, Grobbee DE, Muller M, Emmelot-Vonk M, van der Schouw YT. Serum sex hormone and plasma homocysteine levels in middle-aged and elderly men. Eur J Endocrinol 2006; 155: 887–93.
25. Cohen E, Margalit I, Shochat T, Goldberg E, Krause I. Gender differences in homocysteine concentrations, a population-based cross-sectional study. Nutr Metab Cardiovasc Dis 2019; 29: 9–14.
26. Morris MS, Jacques PF, Selhub J, Rosenberg IH. Total homocysteine and estrogen status indicators in the Third National Health and Nutrition Examination Survey. Am J Epidemiol 2000; 152: 140–48.
27. Hinds HE, Johnson AA, Webb MC, Graham AP. Iron, folate, and vitamin B12 status in the elderly by gender and ethnicity. J Natl Med Assoc 2011; 103: 870–77.
28. Risch M, Meier DW, Sakem B, Escobar PM, Risch C, Nydegger U, et al. Vitamin B12 and folate levels in healthy Swiss senior citizens: a prospective study evaluating reference intervals and decision limits. BMC Geriatr 2015; 15: 82.

29. Stead LM, Au KP, Jacobs RL, Brosnan ME, Brosnan JT. Methylation demand and homocysteine metabolism: effects of dietary provision of creatine and guanidinoacetate. Am J Physiol Endocrinol Metab 2001; 281: E1095–100.

30. Semmler A, Heese P, Stoffel-Wagner B, Muschler M, Heberlein A, Bigler L, et al. Alcohol abuse and cigarette smoking are associated with global DNA hypermethylation: results from the German Investigation on Neurobiology in Alcoholism (GINA). Alcohol 2015; 49: 97–101.

31. Yang B, Liu Y, Li Y, Fan SJ, Zhi XY, LuXX, et al. Geographical distribution of MTHFR C677T, A1298C and MTRR A66G gene polymorphisms in China: findings from 15357 adults of Han nationality. PLoS One 2013; 8: e57917.

32. Qin X, Li J, Cui Y, Liu Z, Zhao Z, Ge J, et al. Effect of folic acid intervention on the change of serum folate level in hypertensive Chinese adults: do methylenetetrahydrofolate reductase and methionine synthase gene polymorphisms affect therapeutic responses? Pharmacogenet Genom 2012; 22: 421–28.

33. Ma C, Li L, Wang X, Hou L,Hou L, Xia L, Yin Y, et al. Establishment of Reference Interval and Aging Model of Homocysteine Using Real-World Data. Front Cardiovasc Med 2022; 9: 846685.

34. Pinto E. Blood pressure and ageing. Postgrad Med J 2007; 83: 109–14.

*Received: January 15, 2023*
*Accepted: April 18, 2023*