Clin. Lab. 2019;65:XXX-XXX

SHORT COMMUNICATION

Reference Intervals of Apolipoprotein E in Healthy Chinese Han Adults

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SUMMARY

Background: **Reference intervals (RIs) of Apo E levels are an important parameter for the clinical evaluation of patient health, and the RIs of serum Apo E could be variable in different population. We plan to establish RIs of apolipoprotein E (Apo E) according to the CLSI EP28-A3 guideline in healthy Chinese Han adults.**
Methods: **Serum Apo E values of 1,206 healthy adults (from 19 to 87 years old) were measured by immunoturbidimetry. The relationship between Apo E and age was analyzed by using Spearman's correlation. The differences between the gender and age groups were compared using Mann-Whitney *U* test/Kruskal-Wallis H test. We calculated recommended nonparametric $Q_{2.5}$ and $Q_{97.5}$ percentile intervals and the 90% confidence intervals (CI) of lower and upper limits to define the age- and gender- related RIs.**
Results: **The level of Apo E was higher in females than males. Apo E was significantly associated with aging in adult females ($r = 0.108$, $p < 0.05$), but not in males ($p = 0.518$). The RIs of Apo E for females were 0.0268 - 0.0619, 0.0247 - 0.0603, and 0.0269 - 0.0658 g/L for 18 - 29, 30 - 59, and ≥ 60 years old, respectively, that for males was 0.0242 - 0.0579 g/L.**
Conclusions: **Our results established the age- and gender-specific RIs of serum Apo E in healthy Chinese Han adults in our laboratory**
(Clin. Lab. 2019;65:xx-xx. DOI: 10.7754/Clin.Lab.2018.181116)

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Short Communication accepted December 29, 2018

KEY WORDS

apolipoprotein E, reference intervals

INTRODUCTION

Apolipoprotein (apo) E, a 34 kDa protein of 299 amino acids, has two structural domains separated by a hinge region. The amino-terminal domain contains the low-density lipoprotein (LDL) receptor binding region, and the carboxyl-terminal domain contains the lipid binding region [1-2]. Apo E was initially described as a lipid transport protein and major ligand for LDL receptors with a role in cholesterol metabolism, cardiovascular disease, hyperlipoproteinemia, and atherosclerosis [3-7]. Many studies found Apo E was a major risk factor (causative gene) for Alzheimer's disease, brain injury, stroke, frontotemporal dementia, Down syndrome, Parkinson's disease, and Lewy body disease [3,8-13].

RIs of Apo E levels are an important parameter for the clinical evaluation of patient health. Several studies in Europe had determined age- and gender-specific RIs for Apo E, but there are significant differences between the RIs [14-16]. Usually, the tested level of serum Apo E will be affected by the detection methods and instruments, geographical location, genotype of Apo E, living standard, ethnicity, and many other factors. Thus, the RIs of serum Apo E could be variable in different populations. Currently our laboratory uses the Apo E RIs (0.029 - 0.053 g/L) provided by the reagent manufacturers. This RI is not an age- and gender-specific RI and may not be accurate for clinical application, so we planned to establish a more specific and reliable RI for Chinese Han population in our laboratory. We measured serum Apo E in a large number of healthy adult subjects and established the RIs of Apo E according to CLSI EP28 - A3 guideline [17].

MATERIALS AND METHODS

Study population

In this study, 1,206 adults of Han ethnicity from Zhejiang province in Eastern China, who were attending their annual health examination in the healthcare center of the First Affiliated Hospital of Zhejiang University, were recruited from April 2016 to October 2018. The health status and laboratory raw data of these participants was determined by a self-report questionnaire. Of the 1,206 subjects, 602 were males between the ages of 19 - 87 years and 604 were females between 18 - 79 years. All participants met the following requirements: no history of cardiovascular disease or diabetes mellitus, no history of autoimmune diseases, not taking corticosteroids, not taking lipid lowering drugs, triglyceride level below 1.70 mmol/L, total cholesterol level below 5.70 mmol/L, low density lipoprotein cholesterol (LDL-c) level below 3.61 mmol/L, high density lipoprotein cholesterol (HDL-c) above 0.91 mmol/L, fasting blood glucose level below 6.16 mmol/L, normal liver and kidney function, and no history of cancers. The ultrasonography results for liver, biliary system, spleen, pancreas, prostate, breast, and uterus were all normal. This study was approved by the Ethics Committee of the First Affiliated Hospital, College of Medicine, Zhejiang University, and written informed consent was obtained from each individual.

Sample collection and Biochemical assay

Venous blood was collected from subjects in a state of quiet and fasting. Serum samples were obtained by centrifugation within 2 hours of initial collection. Biochemical assays of blood samples were completed on the same day. Apo E was measured with a Hitachi 7600 automatic biochemical analyzer (Hitachi Ltd, Tokyo, Japan) by immunoturbidimetry using Zhicheng reagents (Zhicheng, Shanghai, China).

Statistical analysis

All the experimental data was statistically analyzed using SPSS 20.0 software. The reference intervals acquired are expressed as medians and percentiles. Statistical methods recommended by the CLSI EP28-A3 guideline [17] were used to define the RIs. One-sample Kolmogorov-Smirnov tests were used for assessing normality of Apo E level distributions and Kruskal-Wallis H tests were used for comparisons across trending age-groups. Outliers in the selected data were detected by means of the Dixon's Q-test [18]. Mann-Whitney *U* tests were used to compare the differences between gender groups, and Spearman's correlation analyses were applied for correlations between serum Apo E and age in the whole study population. We calculated recommended nonparametric $Q_{2.5}$ and $Q_{97.5}$ percentile intervals and the 90% confidence intervals (CI) of lower and upper limits to define the age- and gender-related RIs. For all analyses, a p-value below 0.05 indicated statistical significance.

RESULTS

Assessment of distribution normality

The data of Apo E in all 1,206 subjects did not show a normal distribution ($p < 0.001$). The data of Apo E for females were significantly higher than that of males ($p < 0.001$), shown in Table 1. When a separate statistical analysis was performed by gender, the data of Apo E also did not show a normal distribution (females $p < 0.001$, males $p < 0.001$), shown in Figure 1.

The age- and gender-specific RIs of Apo E

The selected 1,206 participants were divided into different groups according to gender and age (18 - 29, 30 - 39, 40 - 49, 50 - 59, and ≥ 60 years), shown in Table 2. As shown in Figure 2, the results of Apo E were significantly positively correlated with age ($p = 0.008$) for females, and the results of Apo E were not correlated with age ($p = 0.518$) for males. Serum Apo E levels among males in the five age groups did not show statistically significant differences ($p = 0.125$). The levels of males fluctuated very little and thus were combined into one group. However, female Apo E levels showed statistically significant difference among the five groups ($p = 0.020$). We found there were no statistically significant differences among these three groups: 30 - 39, 40 - 49, and 50 - 59 years, and thus female participants were combined into three groups (18 - 29, 30 - 59, and ≥ 60 years). Figure 3 also showed the trend of age-related medians of Apo E concentration fluctuated very little for males and fluctuated greatly for females. Nonparametric statistical methods were used to calculate RIs according to gender and/or age were shown in Table 3. Apo E levels in females 30 -59 years of age were significantly lower than those of 18 - 29 ($p = 0.004$) and ≥ 60 years ($p < 0.001$) of age. The RIs of Apo E for females were 0.0268 - 0.0619, 0.0247 - 0.0603, and 0.0269 -

Table 1. The gender-specific RIs of Apo E.

Gender	n	Median (g/L)	Lower limit and 90% CI	Upper limit and 90% CI
Female	604	0.0414	0.0254 (0.0239 - 0.0264)	0.0626 (0.0614 - 0.0641)
Male	602	0.0380	0.0242 (0.0232 - 0.0253)	0.0579 (0.0558 - 0.0606)
Total	1,206	0.0397	0.0247 (0.0238 - 0.0254)	0.0614 (0.0599 - 0.0624)

Table 2. The age-specific RIs of Apo E.

Gender	n	Age (years)	Median (g/L)	Lower limit and 90% CI	Upper limit and 90% CI
Female	604				
	105	18 - 29	0.0438	0.0268 (0.0241 - 0.0293)	0.0619 (0.0598 - 0.0639)
	125	30 - 39	0.0392	0.0236 (0.0214 - 0.0256)	0.0629 (0.0569 - 0.0652)
	119	40 - 49	0.0390	0.0240 (0.0221 - 0.0262)	0.0562 (0.0528 - 0.0614)
	119	50 - 59	0.0401	0.0289 (0.0227 - 0.0300)	0.0604 (0.0579 - 0.0622)
	136	≥ 60	0.0449	0.0269 (0.0230 - 0.0311)	0.0658 (0.0634 - 0.0675)
Male	602				
	110	18 - 29	0.0394	0.0256 (0.0245 - 0.0277)	0.0635 (0.0576 - 0.0673)
	115	30 - 39	0.0373	0.0234 (0.0217 - 0.0257)	0.0575 (0.0553 - 0.0632)
	115	40 - 49	0.0367	0.0242 (0.0226 - 0.0255)	0.0546 (0.0514 - 0.0583)
	128	50 - 59	0.0383	0.0237 (0.0204 - 0.0258)	0.0556 (0.0518 - 0.0576)
	134	≥ 60	0.0384	0.0228 (0.0214 - 0.0271)	0.0603 (0.0538 - 0.0639)

Table 3. The age- and gender- specific RIs of Apo E.

Gender	n	Age (years)	Median (g/L)	Lower limit and 90% CI	Upper limit and 90% CI
Female	604				
	105	18 - 29	0.0438	0.0268 (0.0241 - 0.0293)	0.0619 (0.0598 - 0.0639)
	363	30 - 59	0.0394	0.0247 (0.0232 - 0.0259)	0.0603 (0.0577 - 0.0627)
	136	≥ 60	0.0449	0.0269 (0.0230 - 0.0311)	0.0658 (0.0634 - 0.0675)
Male	602	18 - 87	0.0380	0.0242 (0.0232 - 0.0253)	0.0579 (0.0558 - 0.0606)

0.0658 g/L for 18 - 29, 30 - 59, and ≥ 60 years old respectively, that for males was 0.0242 - 0.0579 g/L.

DISCUSSION

Our study establishes the age- and gender- specific RIs of serum Apo E levels in healthy Chinese Han participants according to the CLSI EP28-A3 guideline. This study demonstrates gender differences in RIs of serum Apo E levels, significantly lower serum Apo E levels in males as compared to females, as well as female age differences. The RIs of females 30 - 59 years (0.0247 - 0.0603 g/L) showed lower levels than those of females 18 - 29 (0.0268 - 0.0619 g/L) and ≥ 60 (0.0269 - 0.0658 g/L) years.

As early as 1994, the RI of Apo E was proposed by the study of Gracia V et al. [14]. They obtained the mean Apo E levels in 168 middle-aged subjects randomly selected from general population was 0.0393 - 0.0641 g/L by sandwich enzyme-linked immunosorbent assay (ELISA) using commercially available reagents. With the development and innovation of detection methodology, the study of Schiele F et al. [15] detected the Apo

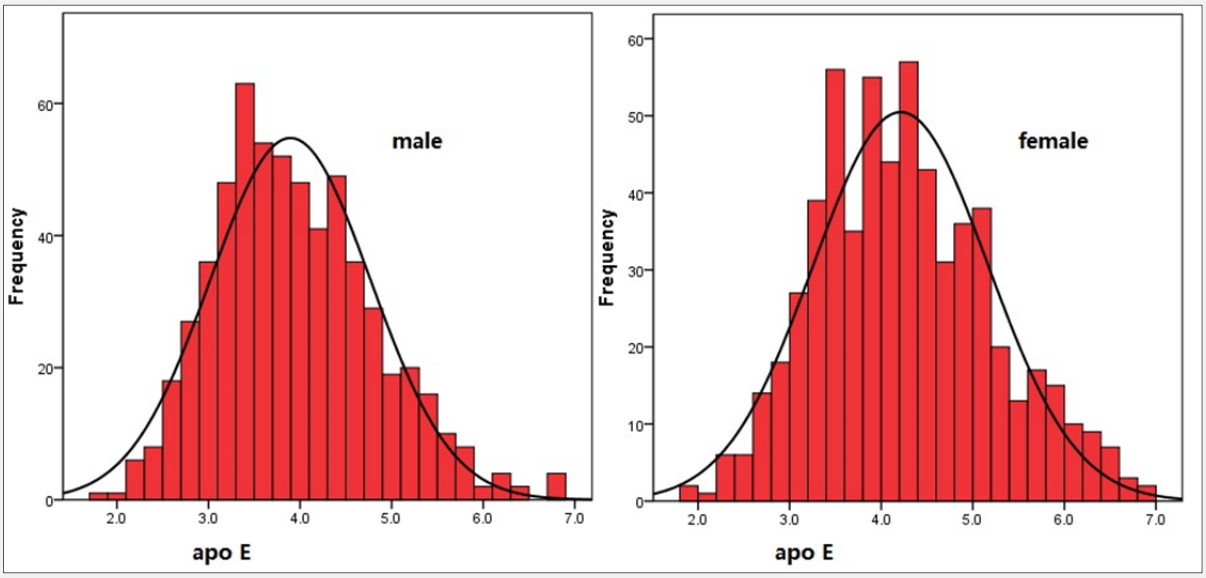

Figure 1. The histograms of Apo E for females and males.

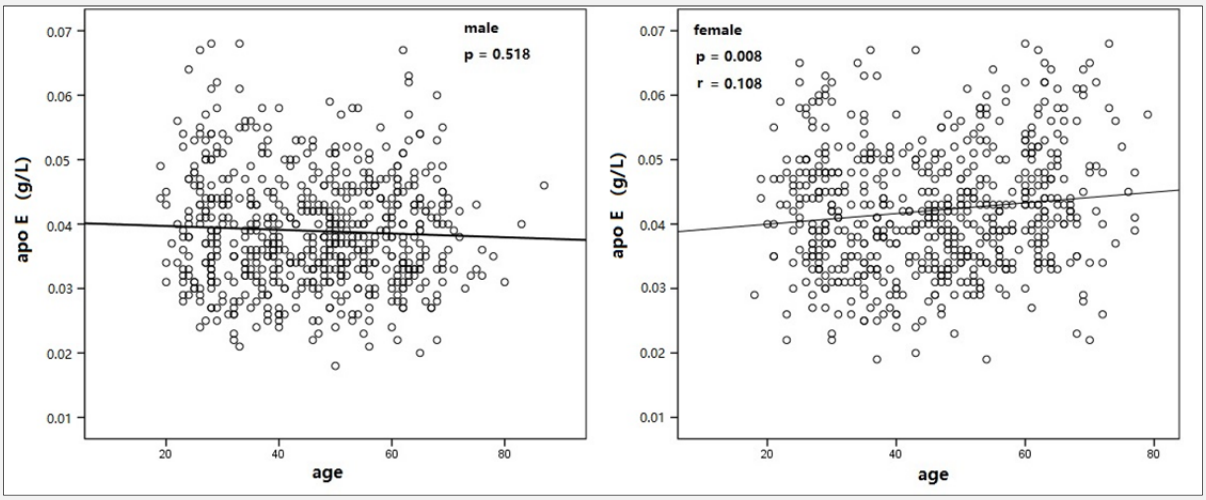

Figure 2. The correlation between Apo E and age for females and males.

E levels of 6,934 healthy subjects from the Apo E Europe Project by immunoturbidimetry and found that age and gender influenced Apo E levels. The levels of males were significantly higher than that of females for those aged between 25 and 44 years, and the levels of males were a plateau levels and the levels of females displayed a linear increase for those aged ≥ 45 years. This result was different from our result, we found the levels of males fluctuated very little among different age groups and that of females fluctuated greatly. RIs of females 30

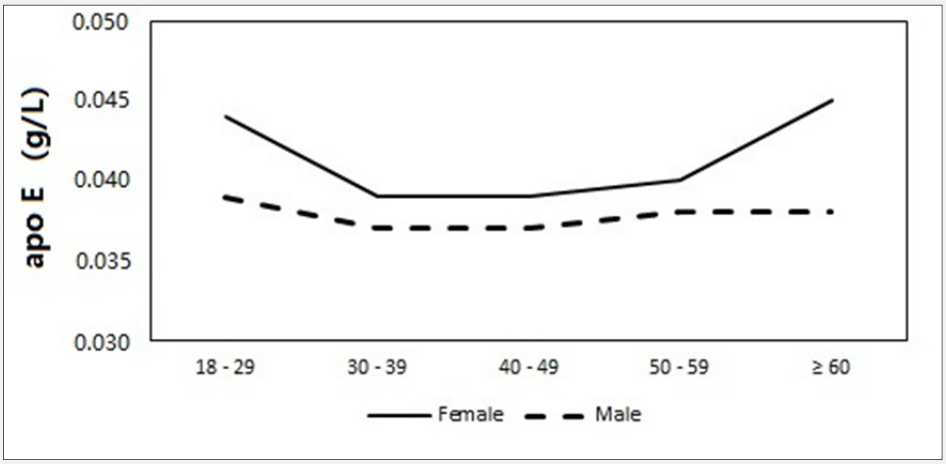

Figure 3. The trend chart of age-related medians of Apo E concentration.

- 59 years of age was lower than those of the other two female groups. In addition, Vincent-Viry M et al. [16] also determined serum Apo E levels by immunoturbidimetry in 4284 subjects from 4 to 71 years of age from the STANISLAS cohort study. The study found that Apo E levels ranged from 0.016 to 0.169 g/L, with a geometric mean +/- standard deviation (SD) values of 0.0466 +/- 0.0138 g/L in the overall sample. Females exhibited higher Apo E concentrations than males in the age of 17 - 26 years. Serum Apo E concentrations were higher in males than in females after those aged > 26 years. There are also large differences between the RIs of their results and ours. The main reason might be that geographical location and ethnicity were different. Apo E is a major risk factor for cardiovascular disease, Alzheimer's disease, and other neurodegenerative disorders [3,4], so the establishment of a reference interval for Apo E levels is needed for the clinical evaluation.

CONCLUSION

In summary, we established age- and gender-related RIs for Apo E and discovered that Apo E tended to change with age in adult females but not in males. There are two limitations in our study. First, this was a single center study based on Chinese Han participants in Zhejiang province. Second, the genotype of Apo E was not considered.

Authors' Contributions:
The study was designed by Y.Z. Experimental data was obtained by Y.Z., Z.Y.L., L.M.F., and G.F.F. Data analyses were performed by Y.Z.. The paper was written by G.F.F. and Y.Y.D. All authors read and approved the final version of the manuscript.

Acknowledgment:
This work was financially supported by grants from the medical and health Foundation of Zhejiang Province, China (2016KYB102) and Education Foundation of Zhejiang Province, China (Y201636519). The funding organization had no role in the design or conduct of this research.

Declaration of Interest:
The authors declare that they have no competing interest.

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