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ORIGINAL ARTICLE

Establishment of a Reference Interval for Urinary Protein Markers for the Healthy Population in East China

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SUMMARY

Background: **The importance of the ratio of creatinine to urinary microalbumin, low-molecular weight protein, and urinary enzymes as urinary markers in patients with chronic kidney disease is widely recognized. However, to date, no reference intervals (RIs) have been established for these markers in East China. The present study aimed to investigate and establish RIs for urinary protein markers in East China's healthy population using the laboratory information system database.**

Methods: **A total of 6,786 healthy individuals were subjected to periodic health examinations in the First Affiliated Hospital of Zhejiang University School of Medicine from January 2022 through December 2023 and were thus included in the study. We used Box-Cox conversion combined with Tukey's method to normalize the data and eliminate outliers. The Mann-Whitney U test was performed to decide on groupings, and a nonparametric method was used to estimate the RI. The upper limit of the RI was set at the 95th percentile of the urinary protein markers.**

Results: **The urinary protein markers were significantly different between males and females, except for retinol binding protein (RBP). The urinary levels of immunoglobulin G (IgG), α1-microglobulin (α1-MG) (female group), transferrin (TRF), N-acetyl-β-D-glucosidase (NAG), RBP/creatinine (Cr), IgG/Cr, mAlb/Cr, TRF/Cr, α1-MG/Cr, and NAG/Cr increased with age. Significant age-related differences in urinary protein marker levels were observed, except for RBP, mALB, and α1-MG (male group). For the significant differences in RIs between age and gender groups, we recommend establishing gender- and age specific RIs for urinary markers.**

Conclusions: **Our study established age- and gender-specific RIs for six urinary protein markers and their ratios to creatinine based on healthy individuals from East China, which was of great significance for kidney disease screening, treatment, and recurrence monitoring.**

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INTRODUCTION

Early kidney damage is usually asymptomatic because of the strong compensatory ability of the kidneys. When patients experience proteinuria, irreversible damage to the kidneys has already occurred, which abrogates the best time for treatment. Therefore, detecting the early signs of kidney function damage is particularly important. The commonly used clinical renal function indicators, such as blood urea and creatinine levels, only in-

crease when renal function is seriously damaged. Therefore, they cannot be used as early monitoring indicators for renal function damage [1].

In recent years, urinary enzymes, represented by urinary N-acetyl-β- D-glucosidase (urinary NAG), have become ideal indicators to detect early kidney disease. NAG in the blood cannot be filtered by the glomeruli, and an increase in urinary NAG indicates damage to the proximal renal tubular epithelial cells, in which ruptured lysosomes release NAG, thus urine NAG is a specific indicator reflecting renal tubular damage [2].

Normally, the glomerular filtration membrane exhibits electrostatic repulsion with a charge selective barrier, and urine microalbumin (UmALB) cannot pass through the glomerular filtration membrane. Various inflammations, metabolic abnormalities, and immune damage can lead to a decrease in negative charges on the filtration membrane, a decrease in electrostatic repulsion, and an increase in urine mALB. Thus, urine mALB is a sensitive indicator for early renal injury [3].

α1-microglobulin (α1-MG) is a small molecular weight protein found in various body fluids, with a molecular weight of 27 kDa. In healthy kidneys, α1-MG can freely pass through the glomerular filtration membrane, and about 99% of it is reabsorbed and decomposed by the proximal renal tubules [4]. When the renal tubules are damaged and their reabsorption capacity decreases, the excretion of urine α1-MG increases. Therefore, an elevated level of urinary α1-MG is an early sign of proximal renal tubular injury [5].

Retinol binding protein (RBP) is a low molecular weight protein with a molecular weight of 21 kDa. Approximately 90% of RBP in normal blood binds to thyroid binding protein and cannot be filtered by the glomerulus; with 10% of RBP being filtered out by the glomerulus and reabsorbed by the renal tubules. Consequently, the urine of healthy individuals has an extremely low RBP level. However, when the glomerular filtration membrane or renal tubular function is impaired, urinary RBP can be significantly elevated. Its extremely small molecular weight and stability in acidic urine mean that RBP is considered the most sensitive indicator of renal proximal tubular injury [6].

Urinary immunoglobulin G (UIgG) and urinary transferrin (UTRF) are considered markers of glomerular dysfunction [7]. IgG and TRF are high molecular weight proteins, with molecular weights of 146 kDa and 80 kDa, respectively. In a physiological state, they cannot pass through the glomerular filtration barrier [8]. Therefore, their presence in urine indicates disruption of the glomerular filtration barrier. Thus, UIgG and UTRF are markers of glomerular injury [8-9].

The reference interval (RI) is a critical basis for clinicians to judge whether a test result indicates health or not, and to make clinical decisions [10]. With the increasingly popular application of urinary protein markers in kidney diseases, appropriate RIs of urinary protein markers are essential for a health evaluation, diagnosis of kidney diseases, therapy monitoring, and prognosis assessment. To date, there have been few studies [11,12] on RIs for urinary protein markers in China, and most hospitals adopt the RI sourced from documents such as health industry guidelines, textbooks, and instructions provided by instrument or reagent manufacturers, which ignores the existence of differences in different countries, regions, seasons, ages, and genders. Personalized RIs have not yet been established in many clinical laboratories in China.

The documents CLSI EP28-A3C published by the Clinical and Laboratory Standards Institute (CLSI) encourage every laboratory to establish its own RI. Our laboratory currently uses the RIs recommended by the reagent manual. In practice, we found that it does not meet the needs of the East China population. Therefore, it is necessary to establish the RIs of urinary protein markers for people in East China. According to the CLSI EP28-A3C guidelines, two methods should be used to establish RIs: direct and indirect sampling methods [13,14]. Establishing RIs by recruiting healthy individuals is a direct sampling method; however, it is costly and time-consuming [15-17]. First described in 1963 [18], indirect sampling must include a large number of healthy individual records from databases, and it is based on data mining techniques. Indirect sampling is cost-effective, faster, easier to perform, and requires fewer material resources compared with direct sampling methods [19,20].

In this study, we planned to use the indirect method to establish RIs of urinary protein markers based on the periodic health examination data of East China population.

MATERIALS AND METHODS

Subjects

Data from the electronic health records of 7,108 individuals who underwent physical examinations at the First Affiliated Hospital of Zhejiang University between January 1st, 2022, and December 31st, 2023, were obtained. Based on the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC)/Reference Intervals and Decision Limits (C-RIDL) protocol [21], the following exclusion criteria were used: 1) a history of acute or chronic diseases, including respiratory diseases, circulatory diseases, digestive diseases, urinary diseases, autoimmune disease, acute and chronic infections, metabolic and nutritional diseases, blood system diseases, endocrine diseases, and cancers; 2) a body mass index (BMI) ≥ 28 kg/m2 or ≤ 18.5 kg/m2; and 3) systolic blood pressure ≥ 160 mmHg or diastolic blood pressure ≤ 100 mmHg. Using the abovementioned exclusion criteria, we finally included 6,786 healthy individuals who did not show significant abnormalities upon physical examination (shown in Figure 1). There were 4,042 males and 2,744 females, with ages ranging from 18 to 90 years.

Ethical approval

This study was approved by the Ethics Committee of the First Affiliated Hospital, Zhejiang University School of Medicine (ethical approval ref: 2022-1071) and was performed in accordance with the Declaration of Helsinki. This study was retrospective in nature, and the results were anonymized; therefore, informed consent for the use of the samples and data was not required.

Measurement of urinary protein markers

The urinary protein markers were measured on a Hitachi Labospect008as automated biochemical system (Hitachi Diagnostics, Tokyo, Japan). Measurements of urinary protein markers were carried out within two hours after collection. Urinary mAlb and α1-MG levels were measured by an immunoturbidimetry method and by using DIALAB reagent (DIALAB, Wiener Neudorf, Austria). The urinary RBP and TRF levels were measured by an immunoturbidimetry method and by using BaiRong reagent (BaiRong Diagnostics, Beijing, China). The urinary IgG and urinary Cr were measured by an immunoturbidimetry method and enzymatic method, respectively, and by using Roche reagent (Roche Diagnostics, Shuzhou, China). The urinary NAG level was measured by the 6'-methyl-2-pyridinoyl-2-(acetylamino)-2-deoxy-1-thio-B-D-glucopyranoside (MPT-NAG) method and by using shenSuo reagent (ShenSuo Diagnostics, Shanghai, China). Since we measured urine protein in random urine, we also calculated the urinary protein markers-to-creatinine ratio to avoid the influence of urine concentration and dilution on urine protein results. Cumulative coefficient of variation (CV) of these urinary protein markers at both the high and low levels was less than 3%.

Statistical analysis

SPSS 25.0 (IBM Corp., Armonk, NY, USA) and MedCalc 20.1 (Applied Math, Mariakerke, Belgium) were used for the statistical analysis. Kolmogorov-Smirnov tests were used to evaluate the normality of the data. Box-Cox transformation was conducted to normalize the data, followed by the application of Tukey's test to eliminate the outliers. The upper limit was P75 plus 1.5 times the interquartile range (IQR), and the lower limit was P25 minus 1.5 times the IQR. All values outside this range were considered outliers. All the subjects were grouped into subgroups according to gender (male and female) and age (< 60 young group and ≥ 60 elderly group, referring to the World Health organization (WHO) age division standard and the traditional Chinese age division standard). A Mann-Whitney U test was used to evaluate whether or not to partition RIs by the age and gender subclasses. Finally, a nonparametric method was used to estimate the 95% distribution of the RIs of urinary protein markers. For urinary protein markers, only high values are of clinical concern. Therefore, we used a one-sided RI. All arrangements were carried out with reference to the CLSI EP28-A3C documents published by The Clinical and Laboratory Standards Institute.

RESULTS

Basic characteristics of the enrolled healthy individuals

According to the inclusion and exclusion criteria, a total of 6,786 healthy individual records (male: n = 4,042; female: n = 2,744) were ultimately included in this study. Table 1 shows the baseline characteristics of all the participants. The average ages of males and females were similar; however, the males had significantly higher BMI, albumin, (ALB), alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), UREA, CR, uric acid (UA), glucose (GLU), and triglyceride (TG) levels compared with the females. Table 2 shows the comparison of common biochemical indicators between younger and elder groups in females and males, respectively. We found that almost all the common biochemical indicators were significantly different between the younger and elder groups. Therefore, we speculated that there may also be some differences in urine markers between older people and younger people.

Distribution of urinary protein markers grouped by gender and age

The distribution of urinary protein markers of healthy individuals according to gender is shown in Table 3. The urinary IgG, α1-MG, TRF, mAlb, NAG, Cr, and α1-MG/Cr values in males were significantly higher than those in females. The RBP/Cr, IgG/Cr, TRF/Cr, and mAlb/Cr values were significantly higher in females than in males. The urinary protein markers were significantly different between males and females, except for RBP, suggesting that we should partition RIs by gender subclass.

Then, we further grouped the urinary protein markers by age (as is shown in Supplementary Table S1). The urinary levels of IgG, α1-MG (female group), TRF, NAG, RBP/Cr, IgG/Cr, mAlb/Cr, TRF/Cr, α1-MG/Cr, and NAG/Cr increased with age. Significant age-related differences in urinary protein marker levels were observed, except for RBP, mALB, and α1-MG (male group), suggesting that we should also partition RIs by age subclass.

Establishment of the gender- and age-specific RIs of urinary protein markers

For the significant differences among age and gender groups, we recommend establishing gender- and age specific RIs for IgG, α1-MG (female), TRF, NAG, RBP/Cr, IgG/Cr, mAlb/Cr, TRF/Cr, α1-MG/Cr, and NAG/Cr. In accordance with the nonparametric methods recommended by the CLSI EP28-A3C and combined with the clinical value of urinary protein markers, the upper reference limit is very important, which was set at the unilateral 95th percentile in this study.

Table 1. Baseline characteristics of 6,786 healthy individuals.

Indicators	Female (n = 2,744)	Male (n = 4,042)	p
Group			
Young	2,331 (84.9%)	3,499 (86.6%)	0.065
Elderly	413 (15.1%)	543 (13.4%)	
Age (years)	50 (43, 55)	49 (43, 55)	0.070
BMI (kg/m^2)	22.89 (21.07, 24.95)	24.95 (23.07, 26.87)	< 0.001
TP (g/L)	72.5 (69.9, 75.3)	72.2 (69.60, 74.9)	< 0.001
Alb (g/L)	45.8 (44.2, 47.4)	46.9 (45.2, 48.6)	< 0.001
ALP (U/L)	62 (50, 77)	66 (56, 78)	< 0.001
ALT (U/L)	14 (11, 20)	22 (16, 31)	< 0.001
AST (U/L)	19 (16, 22)	21 (18, 25)	< 0.001
Urea (mmol/L)	4.96 (4.19, 5.78)	5.35 (4.59, 6.22)	< 0.001
Cr (μmol/L)	57 (52, 62.5)	78 (72, 85)	< 0.001
UA (μmol/L)	269 (235, 308.5)	377 (330, 428)	< 0.001
Glu (mmol/L)	4.75 (4.45, 5.10)	4.86 (4.50, 5.34)	< 0.001
Tch (mmol/L)	4.64 (4.10, 5.25)	4.62 (4.09, 5.18)	0.090
TG (mmol/L)	1.14 (0.82, 1.65)	1.62 (1.15, 2.36)	< 0.001

Table 2. Comparison of biochemical indicators between the young and elderly groups.

Indicators	Female		p	Male		p
	young (n = 2,331)	elderly (n = 413)		young (n = 3,499)	elderly (n = 543)	
TP (g/L)	72.5 (69.9, 75.2)	72.9 (70.4, 75.4)	0.097	72.40 ± 3.95	71.44 ± 4.22	< 0.001
Alb (g/L)	45.8 (44.3, 47.5)	45.5 (43.8, 46.9)	< 0.001	47.1 (45.4, 48.8)	45.5 (43.9, 47.4)	< 0.001
ALP (U/L)	60 (49, 74)	76 (65, 90)	< 0.001	66 (56, 77)	66 (57, 80)	0.032
ALT (U/L)	14 (11, 20)	16 (12, 21)	< 0.001	22 (16, 32)	18 (14, 26)	< 0.001
AST (U/L)	18 (16, 21)	21 (18, 24)	< 0.001	21 (18, 25)	21 (18, 25)	0.528
Urea (mmol/L)	4.88 (4.12, 5.69)	5.34 (4.57, 6.25)	< 0.001	5.29 (4.54, 6.13)	5.89 (5.01, 6.71)	< 0.001
Cr (μmol/L)	57 (52, 62)	60 (54, 66)	< 0.001	78 (72, 85)	79 (70, 87)	0.284
UA (μmol/L)	266 (232, 305)	288 (247, 332)	< 0.001	380 (333, 431)	359 (319, 409)	< 0.001
Glu (mmol/L)	4.73 (4.43, 5.06)	4.96 (4.61, 5.41)	< 0.001	4.84 (4.48, 5.29)	5.03 (4.62, 5.69)	< 0.001
Tch (mmol/L)	4.59 (4.06, 5.21)	4.89 (4.36, 5.47)	< 0.001	4.63 (4.12, 5.20)	4.46 (3.92, 5.06)	< 0.001
TG (mmol/L)	1.09 (0.79, 1.59)	1.40 (1.04, 1.94)	< 0.001	1.67 (1.19, 2.44)	1.40 (0.97, 1.93)	< 0.001

Therefore, we established RIs for urine markers in our laboratory. Since there were no gender and age differences in the RBP RI, we established a single RI < 0.57 mg/L. There was no age difference in the α1-MG male group, and we established RI as < 28.1 mg/L for males, 16.6 mg/L for females < 60 years group, and 19 mg/L for females ≥ 60 years group. The 90% confidence intervals of mAlb RIs in different gender groups were different, but the 95th percentiles were consistent, so we used a single RI < 33 mg/L for mAlb. We established gender- and age-specific RIs for IgG, TRF, NAG, RBP/Cr, IgG/Cr, mAlb/Cr, TRF/Cr, α1-MG/Cr, and NAG/Cr, and the detailed RIs for the urinary protein markers are shown in Supplementary Table S2. The RIs calculated in this study were compared with the RIs currently used in our laboratory (shown in Supplementary

Table 3. Comparison of urinary protein markers after grouping by gender.

Indicators	Male	Female	p
RBP (mg/L)	0.34 (0.23, 0.43)	0.34 (0.23, 0.43)	0.7126
IgG (mg/L)	3.4 (1.7, 5.2)	3.1 (1.5, 4.8)	< 0.001
α1-MG (mg/L)	11.3 (7.3, 16.7)	7.0 (4.8, 10.4)	< 0.001
TRF (mg/L)	0.25 (0.14, 0.43)	0.22 (0.13, 0.38)	< 0.001
mAlb (mg/L)	11 (6, 19)	10 (5, 18)	< 0.001
NAG (U/L)	5 (3, 8)	3 (2, 5)	< 0.001
RBP/Cr (mg/g)	0.19 (0.12, 0.29)	0.27 (0.16, 0.43)	< 0.001
IgG/Cr (mg/g)	1.99 (1.13, 3.21)	2.52 (1.42, 4.11)	< 0.001
α1-MG/Cr (mg/g)	6.85 (4.94, 9.41)	6.66 (4.51, 9.01)	< 0.001
TRF/Cr (mg/g)	0.14 (0.09, 0.25)	0.18 (0.11, 0.29)	< 0.001
mAlb/Cr (mg/g)	5.99 (3.81, 9.75)	7.71 (4.76, 12.67)	< 0.001
NAG/Cr (U/μmol)	2.80 (1.94, 4.11)	2.78 (1.88, 4.04)	0.1738
Cr (μmol/L)	15,044 (10,756, 20,013)	10,259 (6,991, 14,654)	< 0.001

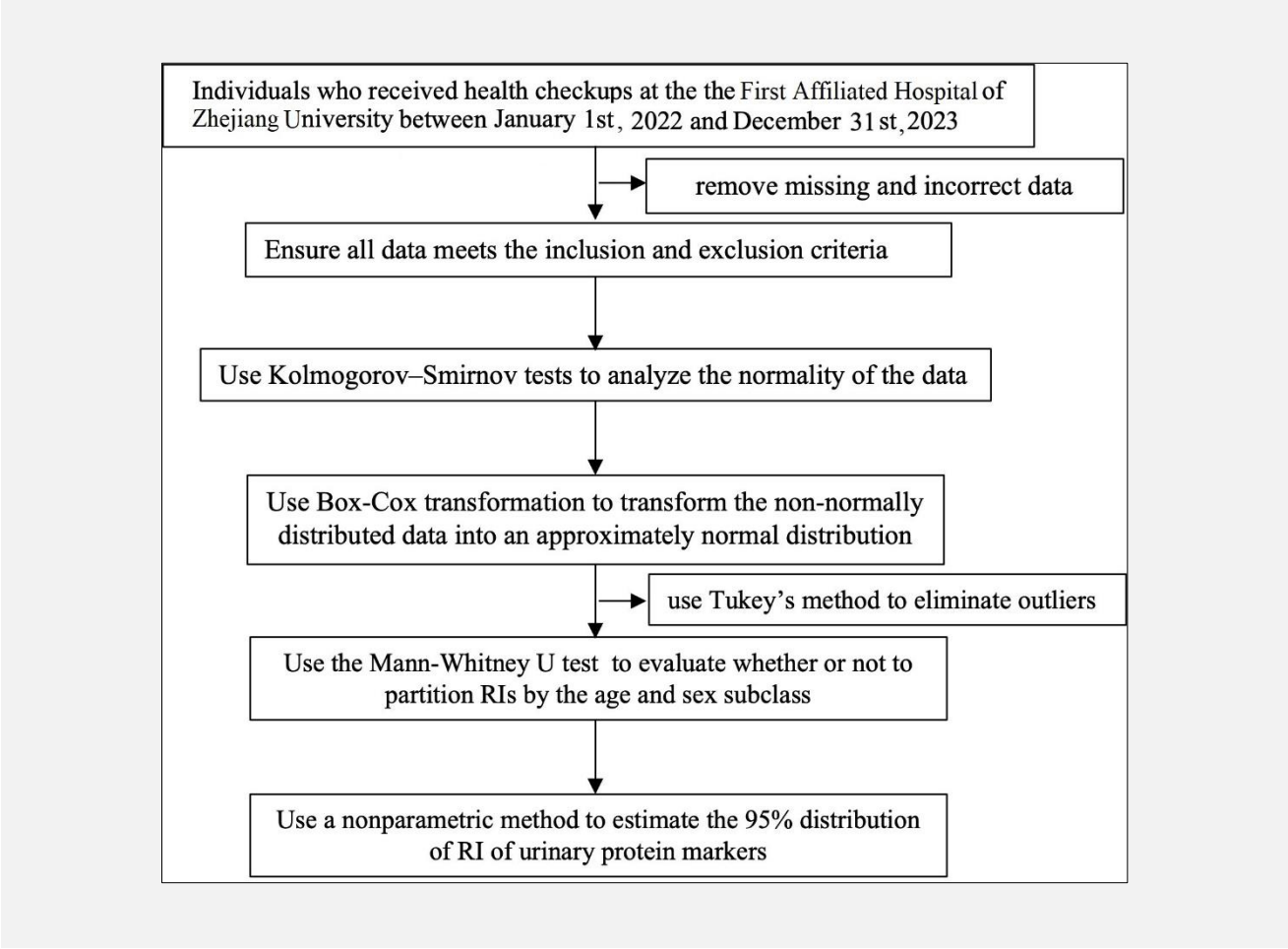

Figure 1. Flowchart.

Establishing reference intervals (RIs) for urinary protein markers based on the Clinical and Laboratory Standards Institute (CLSI) document CLSI EP28-A3C.

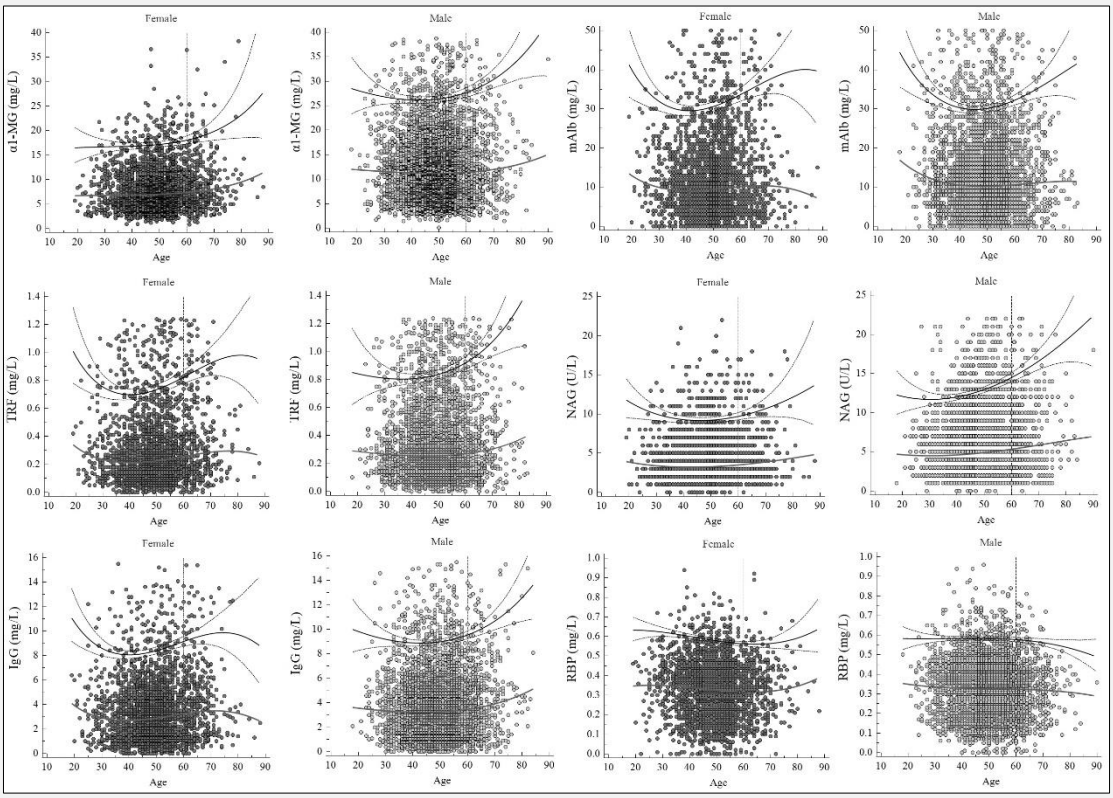

Figure 2. The visualization plot of age and gender-related reference intervals.

The solid points represent females and the hollow points represent males. The vertical dashed lines separate individuals who were over 60 years old. The upper solid line (dashed line) represents the 95th percentile reference interval (90% CI). The middle solid line represents the median.

Table S2). It was found the new RIs for mAlb, α1-MG, and NAG (male) were higher than the RIs currently used in our laboratory, and the new RIs for RBP, IgG, TRF, and NAG (female) were lower than the RIs currently used in our laboratory.

Figure 2 shows the age- and gender-related RI plots of all urinary protein markers. The change of RIs with age can also be directly seen from Figure 2; most indicators changed around the age of 60 years, and we found that the RIs of IgG, TRF, α1-MG (female), NAG, RBP/Cr, IgG/Cr, mAlb/Cr, TRF/Cr, α1-MG/Cr, and NAG/Cr increased significantly with the increase in age.

DISCUSSION

Urine marker detection plays an important role in kidney disease screening, treatment, and recurrence monitoring, and RIs have a great impact on the interpretation of results. However, there are few studies on urinary RIs in Chinese populations of different ages and genders. This study retrospectively analyzed the data and related medical records of the medical examination center of our hospital for 2 years. Through statistical analysis, we pointed out the relationship between the RIs of 6 urine markers (RBP, IgG, TRF, mAlb, α1-MG, NAG) and their ratio to creatinine (RBP/Cr, IgG/Cr, mAlb/Cr, TRF/Cr, α1-MG/Cr, NAG/Cr) and gender/age in East China. We present revised RIs and compare them with the RIs currently used in our laboratory.

Early kidney damage is usually asymptomatic; however, the early signs of kidney function decline are particularly important for diagnosis [4]. In recent years, urinary protein markers have been widely used as indicators of the severity of early kidney damage [6,8]. Therefore, the correct assessment of urinary protein marker levels has important clinical implications for the early diagnosis, treatment, and prognosis of kidney disease. RI is the standard used to make a medical diagnosis, for therapeutic assessment, or for other physiological assessments. Most clinical decision-making processes are based on information provided by laboratory reports. Therefore, providing reliable RIs is essential for clinical laboratories.

Inappropriate RIs for the patients can either cause unnecessary psychological and economic burdens on individuals if they are too narrow or delay early diagnosis if they are too broad. Currently, the RIs used in clinical laboratories mainly come from the reagent manufacturers, the National Clinical Laboratory Procedures, or are found in the scientific literature. However, because of the influence of ethnicity, age, gender, geographical location, and the detection system, methods, and analyzers used, inconsistent test results might be produced. Therefore, a laboratory establishes a local RI that is conducive to the definitive diagnosis and effective treatment of the disease.

The CLSI EP28-A3C documents recommend a direct method to establish the RI [14]. However, such a direct method requires a large workforce and plentiful material resources to develop the RI; therefore, it is challenging to implement it under current laboratory conditions. With the development of information technology, indirect methods to establish the RI using a large amount of data stored in an LIS has also been accepted by EP28-A3C and has been used in many studies [22]. The RI established by the nonparametric method was superior to the RI of the reagent instructions, and thus can better reflect the urinary protein marker levels of healthy individuals in the East China region and can be used as a clinical reference.

Qian Liu et al. [11] reported that the RIs of urinary NAG from Jiangsu Province were < 19.4 U/L (90% CI: 18.0 - 20.3 U/L) for males aged 20 - 59 years, < 22.3 U/L (90% CI: 20.2 - 22.6 U/L) for males aged 60 - 79 years, < 15.7 U/L (90% CI: 15.2 - 16.5 U/L) for females aged 20 - 59 years), and < 21.4 U/L (90% CI: 20.3 - 22.3 U/L) for females aged 60 - 79 years. Qian Zhang et al. [12] established the RI of urinary α1-MG in healthy Tianjin adults aged 20 - 60 as < 26.4 mg/L for males and < 8.6 mg/L for females. Klaus Jung et al. [23] found that the normal RI of the urinary α1-microglobulin-to-creatinine ratio (α1-MG/Cr) was: 1.27 g/mol (11.2 mg/g) creatinine for people aged 18 to 40 and 2.20 g/mol (19.4 mg/g) creatinine for people aged > 40 years. These results differed from those of our study because of regional differences. This further illustrates the necessity of establishing RIs for our laboratory in the East China region. However, there are few detailed and comprehensive studies on the RIs for other urinary protein subjects among Chinese people, and we usually refer to the manufacturer's results. This research investigated the reference intervals of six urinary protein markers for the first time, which will be important for clinicians to make disease diagnoses and assess the effects of therapy.

This study investigated the RIs of urinary protein markers and the RIs of urinary protein markers-to-creatinine ratios. This was because urinary creatinine mainly comes from blood creatinine, which is filtered by the glomerulus and excreted with urine, and the amount of urinary creatinine excreted is basically constant when renal function is normal or only mildly impaired. Therefore, by correcting with the urinary creatinine level, the impact of urine output can be reduced, which could significantly improve the accuracy of the detection of the indicators.

The study of urinary protein markers-to-creatinine ratios in healthy adults showed that there are statistically significant differences between gender and age groups, indicating an increasing trend with age. This is because with age, the functions of various organs in the human body gradually decline, including the glomerular filtration function, resulting in an increase in the amount of urinary protein excreted. Moreover, with increasing age, the density and quality of human muscles gradually decrease, leading to a decline in the level of urinary creatinine excreted [24]. Consequently, this leads to an increase in the urinary protein markers-to-creatinine ratios.

There were some limitations to this study. First, the indirect method used in this study has specific restrictions for the inclusion and exclusion of reference individuals and might include some potentially diseased individuals. Therefore, our study adopted Tukey's test to eliminate the outliers and obtained a data distribution very close to that of healthy population, and we believed that these potentially diseased individuals have little impact on our findings. Second, because of the lack of data on children and older people (above 90 years), we could not establish the RI of urinary protein markers in healthy children and the oldest of old people. Third, our study did not use a multicenter collaborative data collection method, which may limit the generalization of our results.

In summary, we initially established urinary protein marker RIs that were suitable for the healthy East China population, which will help clinicians to diagnose early kidney disease. We recommend that age and gender-specific RIs should be established for IgG, α1-MG, TRF, NAG, Cr, RBP/Cr, IgG/Cr, mAlb/Cr, TRF/Cr, α1-MG/Cr, and NAG/Cr. Using data from individuals who were undergoing periodic health examinations, together with Box-Cox conversion combined with the Tukey's method and a nonparametric method, we developed a reliable indirect method to acquire RIs.

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Ethical Approval:
This study was approved by the Ethics Committee of the First Affiliated Hospital, Zhejiang University School of Medicine (ethics approval ref: 2022-1071). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Data Availability Statement:
The data analyzed in the study are available from the corresponding author on reasonable request.

Declaration of Interest:
The authors state that they have no conflicts of interest.

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