Archives of Razi Institute, Vol. 78, No. 3 (2023) 955-961

DOI: 10.22092/ARI.2022.360017.2530

Original Article

Establishment of Reference Intervals for Renal Function Clinical Chemistry Parameters in an Indonesian Tertiary Hospital

Liana, P[1, 2 *], Olivia, O[3], Fertilita, S[4], Umar, T. P[3]

1. Department of Clinical Pathology, Faculty of Medicine, Universitas Sriwijaya, Mohammad Hoesin General Hospital, Palembang, Indonesia
2. Biomedicine Doctoral Program, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia
3. Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia
4. Department of Histology, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia

Received 14 September 2022; Accepted 18 September 2022
Corresponding Author: pheyliana@fk.unsri.ac.id

Abstract

Reference intervals aid clinical decision-making for clinical chemistry values. Laboratory test results are compared to reference intervals to aid in the diagnosis, therapy, and monitoring decisions. Due to the differences in ethnicity, gender, age, and analytical methods, reference intervals (RIs) vary between populations. This study aimed to establish the reference values for renal function tests in targeted populations in Indonesia. This research was conducted with a cross-sectional observational analytic design. The research sample consisted of medical check-up data from health professionals at Dr. Mohammad Hoesin Hospital in Palembang, Indonesia. The Kolmogorov-Smirnov test was used to determine the normality of data distribution. The RIs were computed using reference limits at the 2.5th and 97.5th percentiles (abnormal distribution) or ±two standard deviations (±2 SD) (normal distribution). The independent t-test (parametric) or Mann-Whitney test was used to compare the RIs of males and females (non-parametric). Males and females had a significant difference (P<0.001) regarding the values of uric acid, urea, and creatinine parameters, requiring the reference intervals to be separated. The following reference intervals were established: uric acid: 230,78-526,99 mol/L for males and 179,03-415.17 mol/L for females, urea: 2,22-4,99 mmol/L for males and 1,78-4,28 mmol/L for females, and creatinine: 61,01-106,99 mol/L for males and 40,67-77,81 mol/L for females. This study defined gender-specific RIs for three renal function test parameters for the adult population of Palembang, Indonesia. The deployment of population-specific RIs may facilitate better laboratory testing.

Keywords: Clinical laboratory, Kidney function tests, Reference values

1. Introduction

Diagnosis is a critical component of clinical decision-making. The diagnostic process entails obtaining a medical history and performing a physical examination. However, laboratory testing is frequently required to elicit further medical information (1). Clinical chemistry examinations are used to determine the concentrations of biological substances, most often utilizing blood specimens (serum or plasma) to ascertain the health status. Renal function tests (such as uric acid, creatinine, and urea) are among the common types of examination (2).

Clinical chemistry reference intervals serve as auxiliary tools for clinical decision-making by differentiating healthy and ill people. Since laboratory results are interpreted based on comparison with this reference interval, the quality of the reference interval plays a crucial role in the quality of interpretation results (3).

Clinical laboratories frequently employ manufacturer-specified reference intervals or those available in textbooks. However, this has become less reliable since it often has not corresponded to the local population (4).

Reference intervals may vary among subpopulations due to the biological variation caused by ethnic, gender, and age discrepancies. Laboratory-specific variations in sampling technique, analysis instruments, and result interpretation may also impact the used reference intervals. Physiological factors, such as immunological factors, puberty, pregnancy, menopause, and aging can also contribute to differences in laboratory measurement results (5). Since the instrumentation, methodology, reagents, and population of each clinical laboratory are unique, it is advised that each clinical laboratory establishes its reference interval. It is beneficial to improve illness diagnosis, staging, therapy monitoring, the quality of clinical trials, and medical research in particular (6).

The use of reference intervals generated and verified in communities outside the deployment region in medical care may have a detrimental effect on patient clinical management (6). The concentrations of renal function tests, including uric acid, urea, and creatinine, may differ among people from various studied regions and also when compared to the standards of the manufacturer (2). As a result, research on the uric acid, urea, and creatinine reference intervals in certain populations is necessary. In this regard, the present study aimed to develop reference values for renal function tests and ascertain any discrepancies between published and local reference ranges.

2. Materials and Methods

2.1. Study Overview

This laboratory-based observational study employed a cross-sectional design and was conducted at Dr. Mohammad Hoesin General Hospital, Palembang, South Sumatra, Indonesia.

2.2. Study Population

The sample size was determined following the Clinical and Laboratory Standard Institute criteria for examining regional within-country diversity, which recommended a minimum of 240 persons (120 male and 120 female) (7). The population consisted of health workers aged 18-60 years old who had resided in South Sumatra for at least 6 months before the start of this study. After the provision of written informed consent, participants were asked to complete a questionnaire.

When one or more of the following conditions were met, participants were excluded: (1) diabetics treated with oral/insulin drugs, (2) alcohol drinkers, (3) history of chronic kidney/liver disease, (4) hospitalization for 1 month before sample collection, (5) blood donation in the previous 3 months, (6) Infection with hepatitis B virus and/or hepatitis C or human immunodeficiency virus (HIV), (7) history of participation in research involving medical products or dietary supplements in the previous 12 months, (8) pregnant mothers, and (9) women who gave birth in the previous year.

2.3. Specimen Collection and Processing

Blood samples were drawn from suitable donors from October 2020 to January 2021. After specimen acquisition, they were labeled with the study sample numbers. Afterward, approximately 5 ml of blood was collected from each participant in the red-top tubes with clot activators (9 mL) after they underwent a minimum of 10-h fasting period. Samples are screened for HIV, hepatitis B surface antigen, hepatitis C virus, and pregnancy.

The blood specimens were allowed to clot at room temperature for approximately 30-60 min. Subsequently, the specimens were centrifuged at 1,200 g for 15 min using the Hettich Rotina 380 Benchtop Centrifuge (Hettich Marketing- und Vertriebs, Germany). After centrifugation, 500 µL serum was transferred to the cryotube. Hemolytic, lipemic, or icteric samples were rejected. All of the specimens were examined promptly after the completion of sample collection.

Three parameters of renal function were determined, namely urea, creatinine, and uric acid. All assays were performed following the standard operating procedures. The Architect C8000, AS1242 series (Abbott

Diagnostics, USA) was used to test renal function. Uric acid (Lot 70256UN19), urea (Lot 28795UN19-01729), and creatinine (Lot 90628Y600) were determined using the uricase, Berthelot, and Jaffe techniques.

2.4. Statistical Analysis

Descriptive (univariate) analyses outline both continuous and discrete variables. Continuous variables were presented as means and standard deviations (SD). Meanwhile, categorical variables were expressed as numerical values. Kolmogorov-Smirnov test was performed to determine the normality of uric acid, urea, and creatinine levels.

The bivariate analysis evaluated the difference between the male and female groups. Moreover, an independent t-test was performed for the parametric analysis. In addition, the Mann-Whitney U test was utilized for non-parametric analysis. The reference intervals (RIs) were determined only if there were a statistically significant difference between the two genders. If there were no statistically significant differences, the reference intervals were pooled. The RIs were determined from the RIs range from ± two standard deviations (±2 SD) when the data were normally distributed. However, the RIs were taken from the percentile 2.5-97.5 value if the data were not normally distributed. All statistical analysis was performed in the SPSS software (version 25, IBM Corp., Armonk, NY, USA) or Medcalc (version 19.6.3, MedCalc Software bv, Ostend, Belgium). Statistical significance was defined as a *P* value of less than 0.05.

3. Results

In total, 300 records containing the renal function parameters (uric acid, urea, and creatinine) were gathered via the collection of medical check-up data of the health professionals of the hospital. After a quantile-quantile plot inspection, 12 samples that were regarded outliers were discarded and 288 samples remained.

The distribution of research participants is summarized in table 1. Slight disparities in the gender of participants were discovered, as the female gender was more prevalent in this study (female: male ratio=1.23:1). Female participants also possessed an older median age (36 vs. 33 years) with a lower body mass index (23.52 vs. 25.02) and waist circumference (83.82 cm vs. 92 cm). Regardless of gender, the most prevalent blood type among participants was O, followed by A, B, and AB.

Male participants had a median uric acid value of 413.98 µmol/L (350.93-450.56 µmol/L), which was significantly higher than that of female participants which was 272.42 µmol/L (223.75-320.0 µmol/L). Similar findings are applied to urea as the median urea value for males and females were 3.2 mmol/L (2.91-4.00 mmol/L) and 2.91 mmol/L (2.54-3.63 mmol/L), respectively. Due to the normal distribution of creatinine values, mean values were picked, which revealed a higher number in male participants, compared to the female participants (83.68±11.32 µmol/L vs. 58.32±9.00 µmol/L). Since the comparison test revealed substantial variations in uric acid, urea, and creatinine values between male and female groups, the reference intervals for males and females were processed separately. In particular, reference intervals for renal function tests demonstrated a similar trend, with males having higher values (Table 2).

Table 1. Characteristics of Data Based on Sex, Age, Body Mass Index, Waist Circumference and Blood Type

Characteristics		Overall	Male	Female
Sex		288 (100%)	129 (44,8%)	159 (55,2%)
Blood Type	A	83 (28,8%)	37 (12,84%)	46 (15,96%)
	B	68 (23,6%)	25 (8,67%)	43 (14,93%)
	AB	28 (9,7%)	13 (4,5%)	15 (5,2%)
	O	98 (34%)	47 (16,3%)	51 (17,7%)
	Unknown	11 (3,8%)	7 (2,41%)	4 (1,39%)
Age		35 (18-57)[a]	33 (18-57)[a]	36 (20-57)[a]
Body Mass Index		24,44 ± 4,20[b]	25,02 ± 3,85[b]	23,52 (10,42-36,51)[b]
Waist Circumference		86,71 ± 10,63[b]	92 (69-110)[a]	83,82 ± 10,59[b]

Note: Data was presented as (a) median (range) for abnormal distribution, (b) mean ± SD for normal distribution

Table 2. Reference intervals for renal function test

	Male (M)	Female (F)	P-value (normality)	P-value (comparison)
Uric acid (µmol/L)				
Mean (SD)	401.56±71.90	279.10±59.71		
Median (IQR)	413.98 (350.93-450.56)	272.42 (223.75-320.0)	M: 0.041	<0.001
Reference intervals (RIs)	230,78-526,99	179,03-415.17	F: 0.050	
90% CI upper	510.93-578.14	173.08-195.09		
90% CI lower	208.77-280.74	386.02-440.15		
Urea (mmol/L)				
Mean (SD)	3.45±0.69	3.05±0.68		
Median (IQR)	3.2 (2.91-4.00)	2.91 (2.54-3.63)	M: <0.001	<0.001
Reference intervals (RIs)	2.22-4.99	1.78-4.28	F: <0.001	
90% CI upper	2.18-2.54	1.45-1.81		
90% CI lower	4.72-5.09	4.36-4.72		
Creatinine (µmol/L)				
Mean (SD)	83.68±11.32	58.32± 9.00		
Median (IQR)	83.9 (75.28-92.8)	57.82 (50.7-64.64)	M: 0.200	<0.001
Reference intervals (RIs)	61,01-106,99	40,67-77,81	F: 0.200	
90% CI upper	58.64-64.33	38.63-42.70		
90% CI lower	103.01-108.71	73.93-78.00		

4. Discussion

Results of the present study revealed a statistically significant difference between male and female participants in terms of all the performed renal function tests. This phenomenon could be attributed to the discrepancy in estimated glomerular filtration rates between males and females, as determined by the Modification of Diet in Renal Disease or the Mayo Clinic Quadratic Equation formula. It should be mentioned that the differences exist across all age decades (8). Based on these findings, reference interval determinations were conducted separately according to gender.

Males and females had significantly different uric acid concentrations (P<0.001). This result is similar to those of previous studies performed in Ethiopia and India, which indicated that the values were higher for males (9, 10). It is believed to be caused by female sex hormones (estrogen), which regulate the expression or function of uric acid transporters, specifically ABCG2 and SLC2A9. Estrogen and progesterone hormones are also thought to have diuretic or uricosuric properties, lowering uric acid levels (11).

There were significant disparities between male and female participants regarding urea levels. The result of this study was consistent with those of earlier research conducted in Ethiopia, Zimbabwe, Sudan, and China (9, 12-14). The underlying cause generating this discrepancy is the distinction in protein turnover between the genders. Additionally, greater calorie and protein consumption of males (urea is the disposal product of amino acids) contribute to the overall increase in blood urea concentration (13).

The difference between males and females in terms of creatinine values was statistically significant at P<0.001. The significant findings in this study are consistent with those of prior research carried out in Ethiopia, Zimbabwe, and Iran, in all of which, a P value of < 0.001 was considered statistically significant (9, 14, 15). Males had a higher serum creatinine level, compared to females, which might be explained by males having more muscle mass (since creatinine is mainly carried to the muscles for energy transfer in the form of creatine phosphate and then stored as a waste product) and females having a lower creatinine production rate (16).

In this study, reference intervals of uric acid, urea, and creatinine in males were higher than those in females. Similar results were also obtained in previous studies performed in Ethiopia, China, Saudi Arabia, India, and Russia (9, 17-20). These differences are based on gender differences in kidney size and weight as well as biological, metabolic, and hemodynamic processes (21). The comparison results are summarized in table 3.

Variations in reference intervals can be caused by differences in the population, analytical tools, and the used clinical chemistry examination methods. Ethnicity, genetics, demographics, culture, lifestyle, social environment, nutrition/food, disease distribution, and seasonal differences are some of the factors that can affect the reference intervals of healthy individuals (9).

Several factors may affect plasma uric acid levels, such as genetics and nutritional factors. Hereditary aspects may influence the excretory system, affecting the uric acid equilibrium of the body. According to a study, Asians possessed genetic polymorphisms in the uric acid transporter ABCG2 (which is involved in the excretion of uric acid from kidneys via urine). This phenomenon may increase their risk of hyperuricemia, gout, and early-onset gout, compared to Europeans, Americans, and Africans (22). This is consistent with the findings of the present investigation, which indicated higher uric acid reference intervals than those reported in Ethiopia and Russia (9, 18). Consumption of meat, shellfish, alcohol, and fructose foods varies between populations, resulting in the variability of uric acid concentrations. High-purine food consumption may increase blood uric acid levels by 1-2 mg/dL, and it is observable in Asian populations, such as the Chinese (11, 23).

Nutritional status, ethnicity, and methods of clinical chemistry examination may play a role in the variability of urea and creatinine levels. Moreover, food intake, especially protein, which varies in each population can cause variations in urea and creatinine values between populations. Western populations have higher protein intake, especially from meat, since their socioeconomic status tends to be higher, compared to other populations (24). However, the populations studied in this research showed lower reference values, compared to other studies (9,17–20). It can be associated with low protein and calorie intake in Indonesia, especially from the meat source (25). These differences among populations have led research and international guidelines to recommend that local laboratories should establish reference intervals for each population (9).

Table 3. Comparison of Reference Intervals of This Study against Other Studies

Parameter	Sex	Ethiopia (9) Mindray BS-200E	Russia (20) AU5800 *analyzer*	China (17) AU5800 *analyzer*	Saudi Arabia (18) Abbott Architect c16000	India (19) UnicelDxC 800 *autoanalyzer*	This study
Uric Acid (µmol/L)	Male	160,6-410,41	244-478	247-540	223-444	248-509	230,78-526,99
	Female	124,91-350,93	153-366	178-406	148-321	159-404	179,03-415.17
Urea (mmol/L)	Male	2-7,15	3,1-7,9	3,1-7,6	2,8-7,3	2,2-6,0	2,22-4,99
	Female	1,66-6,44	2,3-6,2 (<45 y/o) 2,8-8,4 (≥45 y/o)	2,4-6,4 (20-49 y/o) 3,0-7,7 (50-64 y/o)	2,1-6,4	1,9-5,1 (<45 y/o) 2,4-6,7 (≥45 y/o)	1,78-4,28
Creatinine (µmol/L)	Male	42,44-99,91	65-102	57-102	66-111	58-95	61,01-106,99
	Female	41,56-96,38	51-79	42-73	50-74	35-74	40,67-77,81

The limitation of the study was that it could not include children and older adults to establish reference intervals. Results of this study cannot conclusively determine if age group partitioning was necessary for renal parameter reference ranges. Furthermore, since dietary patterns, particularly protein intake and menopausal state in females, were not evaluated in this study, there might have been differences in renal parameter values. Additionally, a smaller sample size, compared to other research might have imposed restrictions on this study.

This study defined gender-specific RIs for three renal function test measures (uric acid, urea, and creatinine) for adoption in the adult population of Palembang, Indonesia. The deployment of population-specific RIs will facilitate the investigation and interpretation of laboratory test findings; hence, increasing the quality of healthcare services in the region. The authors urge physicians and researchers to use these RIs to perform studies in similar regions to strengthen the test results analysis.

Authors' Contribution

Study concept and design: P. L., O., and S. F.
Acquisition of data: P. L. and O.
Analysis and interpretation of data: P. L., O., and T. P. U.
Drafting of the manuscript: P. L., O., S. F., and T. P. U.
Critical revision of the manuscript for important intellectual content: P. L. and T. P. U.
Statistical analysis: P. L., O., and T. P. U.
Study supervision: P. L. and S. F.

Ethics

The Research Ethics Committee of the Faculty of Medicine, Sriwijaya University, granted the ethical approval for this study (Approval number: 178-2021).

Conflict of Interest

The authors declare that they have no conflict of interest.

Grant Support

This work was supported by the Sriwijaya University Faculty of Medicine Research Grant (Grant number: 0336/UN9.FK/TU.SK/2020).

References

1. Tadeu BT, Geelhoed D. "This thing of testing our blood is really very important": a qualitative study of primary care laboratory services in Tete Province, Mozambique. Int J Equity Health. 2016;15(1):133.
2. Cheneke W, Demie T, Getachew A, Bekele S, Eskinder K, Sahlemariam Z, et al. Reference interval for clinical chemistry test parameters from apparently healthy individuals in Southwest Ethiopia. Ethiop J Lab Med. 2018;5(5):62-9.
3. Martinez-Sanchez L, Marques-Garcia F, Ozarda Y, Blanco A, Brouwer N, Canalias F, et al. Big data and reference intervals: rationale, current practices, harmonization and standardization prerequisites and future perspectives of indirect determination of reference intervals using routine data. Adv Lab Med. 2021;2(1):9-16.
4. Mahato R, Singh R, Dutta A, Ichihara K, Lamsal M. Reference intervals (RIs) of lipid parameters for Nepalese population. Int J Appl Sci Biotechnol. 2018;6(4):366-72.
5. Sikaris KA. Physiology and its importance for reference intervals. Clin Biochem Rev. 2014;35(1):3-14.
6. Ayemoba O, Okeji N, Hussain N, Umar T, Ajemba-Life A, Kene T, et al. Reference intervals of common clinical biochemistry analytes in young Nigerian adults. PLoS One. 2021;16(3):e0247672.
7. Ozarda Y, Ichihara K, Barth JH, Klee G, Committee on Reference I, Decision Limits IFfCC, et al. Protocol and standard operating procedures for common use in a worldwide multicenter study on reference values. Clin Chem Lab Med. 2013;51(5):1027-40.
8. Lippi G, Tessitore N, Montagnana M, Bedogna V, Salvagno GL, Targher G, et al. Influence of age and gender variations on glomerular filtration rate estimated by the MCQE formula. Biochemia Medica. 2009;19(1):81-6.
9. Abebe M, Melku M, Enawgaw B, Birhan W, Deressa T, Terefe B, et al. Reference intervals of routine clinical chemistry parameters among apparently healthy young adults in Amhara National Regional State, Ethiopia. PLoS One. 2018;13(8):e0201782.

10. Das M, Borah NC, Ghose M, Choudhury N. Reference Ranges for Serum Uric Acid among Healthy Assamese People. Biochem Res Int. 2014;2014:171053.
11. Karwur FF, Pujiastuti DR. Review Article: uric acid homeostasis and disturbances. Folia Medica Indonesiana. 2017;53(4):292-8.
12. Abeadalla AA, Bashir AA, Abdalla IM, Ali IA, Awad KM, Mohmed AA, et al. Normal reference value of adult Sudanese serum creatinine and urea in Khartoum state. Int J Health Sci Res. 2018;8(6):19-24.
13. Liu Q, Wang Y, Chen Z, Guo X, Lv Y. Age- and sex-specific reference intervals for blood urea nitrogen in Chinese general population. Sci Rep. 2021;11(1):10058.
14. Samaneka WP, Mandozana G, Tinago W, Nhando N, Mgodi NM, Bwakura-Dangarembizi MF, et al. Adult Hematology and Clinical Chemistry Laboratory Reference Ranges in a Zimbabwean Population. PLoS One. 2016;11(11):e0165821.
15. Ghasemi A, Azimzadeh I, Zahediasl S, Azizi F. Reference values for serum creatinine with Jaffe-compensated assay in adult Iranian subjects: Tehran Lipid and Glucose Study. Arch Iran Med. 2014;17(6):0-.
16. Singh P, Khan S, Mittal R. Renal function test on the basis of serum creatinine and urea in type-2 diabetics and nondiabetics. Bali Med J. 2014;3(1):11-4.
17. Borai A, Ichihara K, Al Masaud A, Tamimi W, Bahijri S, Armbuster D, et al. Establishment of reference intervals of clinical chemistry analytes for the adult population in Saudi Arabia: a study conducted as a part of the IFCC global study on reference values. Clin Chem Lab Med. 2016;54(5):843-55.
18. Evgina S, Ichihara K, Ruzhanskaya A, Skibo I, Vybornova N, Vasiliev A, et al. Establishing reference intervals for major biochemical analytes for the Russian population: a research conducted as a part of the IFCC global study on reference values. Clin Biochem. 2020;81:47-58.
19. Shah SAV, Ichihara K, Dherai AJ, Ashavaid TF. Reference intervals for 33 biochemical analytes in healthy Indian population: C-RIDL IFCC initiative. Clin Chem Lab Med. 2018;56(12):2093-103.
20. Xia L, Chen M, Liu M, Tao Z, Li S, Wang L, et al. Nationwide Multicenter Reference Interval Study for 28 Common Biochemical Analytes in China. Medicine (Baltimore). 2016;95(9):e2915.
21. Belgüzar K. Gender differences in kidney function and health outcomes. AIMS Med Sci. 2019;6(3):179-80.
22. Butler F, Alghubayshi A, Roman Y. The Epidemiology and Genetics of Hyperuricemia and Gout across Major Racial Groups: A Literature Review and Population Genetics Secondary Database Analysis. J Pers Med. 2021;11(3).
23. Li R, Yu K, Li C. Dietary factors and risk of gout and hyperuricemia: a meta-analysis and systematic review. Asia Pac J Clin Nutr. 2018;27(6):1344-56.
24. Alles B, Baudry J, Mejean C, Touvier M, Peneau S, Hercberg S, et al. Comparison of Sociodemographic and Nutritional Characteristics between Self-Reported Vegetarians, Vegans, and Meat-Eaters from the NutriNet-Sante Study. Nutrients. 2017;9(9).
25. Mutalazimah M, Wijaya YA, Suswardany DL. Energy, protein intake and mid-upper arm circumference in pregnant women in boyolali regency, Indonesia. Mal J Med Health Sci. 2020;16:77-83.