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RESEARCH

REFERENCE INTERVALS OF CREATININE AND GLYCATED HEMOGLOBIN PARAMETERS FOR THE BRAZILIAN ADULT POPULATION: NATIONAL HEALTH SURVEY

INTERVALOS DE REFERÊNCIA DE PARÂMETROS DE CREATININA E HEMOGLOBINA GLICOSILADA PARA A POPULAÇÃO ADULTA BRASILEIRA: PESQUISA NACIONAL DE SAÚDE

INTERVALOS DE REFERENCIA DE LOS PARÁMETROS CREATININA Y HEMOGLOBINA GLICOSILADA PARA LA POBLACIÓN ADULTA BRASILEÑA: PESQUISA NACIONAL DE SAÚDE

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ABSTRACT

Objective*:* to estimate reference intervals (RIs) of creatinine and glycated hemoglobin (HbA1c) in the Brazilian adult population. Methods*:* a cross-sectional study, using the National Health Survey (Pesquisa Nacional de Saúde, PNS) database, between 2014-2015, consisting of 8,952 adults. To establish RIs, exclusion criteria were applied, outliers were removed and stratification was performed. After these procedures, the sample consisted of 2,723 adults for HbA1c and 2,738 adults for creatinine. Differences were evaluated by means of the Mann Whitney and Kruskal Wallis tests (p≤0.05). **Results***:* men (RI: 0.69-1.25; median: 0.95 mg/dL) had higher RIs for creatinine than women (RI: 0.53-1.05; median: 0.74 mg/dL) and higher lower limit (LL) values and median HbA1c (male: RI: 4.55-5.97; median: 5.3%; female: RI: 4.49-5.97; median: 5.20%) (p≤0.05). In women, the RIs for creatinine were higher in the age groups between 45 and 59 years old (RI: 0.55-1.04; median: 0.77 mg/dL) and from 60 years old (RI: 0.54-0.98; median: 0.77 mg/dL (p≤0.05). For HbA1c, men had higher RIs from age 60 (RI: 4.65-6.07; median: 5.44%) and women from 45 years old (45-59: RI: 4.61-6.05; median: 5.40%; and 60 years old or more: RI: 4.82-6.03; median: 5.50%) (p≤0.05). For creatinine, lower RI LLs and more prominent medians were observed in white-skinned adults (RI: 0.56-1.19; median: 0.85%) when compared to brown-skinned (RI: 0.55-1.19; median: 0.84%) (p≤0.05). **Conclusion***:* appropriate RIs make it possible to unveil the health conditions of Brazilian adults and can support proper identification of chronic kidney disease and diabetes.

Keywords: Reference Values; Creatinine; Glycated Hemoglobin A; Health Surveys; Risk Factors; Brazil.

RESUMO

***Objetivo*:** *estimar intervalos de referência (IR) de creatinina e hemoglobina glicosilada (HbA1c) na população adulta brasileira.* ***Métodos*:** *estudo transversal, utilizando na base de dados Pesquisa Nacional de Saúde (PNS), entre 2014-2015, composta por 8.952 adultos. Para estabelecer IR, aplicaram-se critérios de exclusão, removeram-se outliers e foi feita estratificação. Após esses procedimentos, a amostra constitui-se de 2.723 adultos para HbA1c e de 2.738 adultos para creatinina. Avaliaram-se diferenças pelos testes Mann Withney e Kruskal Wallis (p≤0,05).* ***Resultados*:** *homens (IR 0,69-1,25; mediana 0,95 mg/dL) apresentaram maiores IR para creatinina que mulheres (IR 0,53-1,05; mediana 0,74 mg/dL) e tiveram maiores valores de limites inferiores (LI) e mediana de HbA1c (sexo masculino: IR: 4,55-5,97; mediana 5,3%; sexo feminino: IR 4,49-5,97; mediana 5,20%) (p≤0,05). Nas mulheres, IR para creatinina foram mais elevados entre 45 a 59 anos (IR: 0,55-1,04; mediana 0,77 mg/dL) e a partir dos 60 anos (IR: 0,54-0,98; mediana 0,77 mg/dL (p≤0,05). Para HbA1c, homens apresentaram IR mais elevados a partir de 60 anos (IR 4,65-6,07; mediana 5,44%) e mulheres a partir de 45 anos (45 a 59 anos: IR 4,61-6,05; mediana 5,40%; e 60 anos ou mais: IR 4,82-6,03; mediana 5,50%) (p≤0,05). Para creatina, foram observados menores LI dos IR e mediana mais proeminente nos adultos de raça/cor branca (IR: 0,56-1,19; mediana 0,85%) em comparação com a parda (IR: 0,55-1,19; mediana 0,84%) (p≤0,05).* ***Conclusão*:** *IR próprios possibilitam desvelar as condições de saúde dos adultos brasileiros e podem subsidiar a identificação adequada de doença renal crônica e diabetes.*

Palavras-chave: *Valores de Referência; Creatinina; Hemoglobina A Glicada; Inquéritos Epidemiológicos; Fatores de Risco; Brasil.*

RESUMEN

Objetivo: *estimar los intervalos de referencia (IR) de creatinina y hemoglobina glicosilada (HbA1c) en la población adulta brasileña.* ***Métodos:*** *estudio transversal, utilizando la base de datos Pesquisa Nacional de Saúde (PNS), entre ww2014-2015, compuesto por 8.952 adultos. Para establecer la IR, se aplicaron criterios de exclusión, se eliminaron los valores atípicos y se realizó una estratificación. Tras estos procedimientos, la muestra estaba formada por 2.723 adultos para la HbA1c y 2.738 adultos para la creatinina. Las diferencias se evaluaron mediante las pruebas de Mann Withney y Kruskal Wallis (p≤0,05).* ***Resultados:*** *los hombres (IR 0,69-1,25; mediana 0,95 mg/dL) tenían un IR de creatinina más alto que las mujeres (IR 0,53-1,05;*

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*mediana 0,74 mg/dL) y presentaban valores de límite inferior (LI) y mediana de HbA1c más altos (hombre: IR: 4,55-5,97; mediana 5,3%; mujer: IR 4,49-5,97; mediana 5,20%) (p≤0,05). En las mujeres, los IR para la creatinina fueron mayores entre los 45 y los 59 años (IR: 0,55-1,04; mediana 0,77 mg/dL) y a partir de los 60 años (IR: 0,54-0,98; mediana 0,77 mg/dL (p≤0,05). En cuanto a la HbA1c, los hombres mostraron una IR más alta a partir de los 60 años (IR 4,65-6,07; mediana 5,44%) y las mujeres a partir de los 45 años (45 a 59 años: IR 4,61-6,05; mediana 5,40%; y 60 años o más: IR 4,82-6,03; mediana 5,50%) (p≤0,05). En el caso de la creatina, observamos un menor LI de los IR y una mediana más prominente en los adultos blancos (IR: 0,56-1,19; mediana 0,85%) en comparación con los adultos morenos (IR: 0,55-1,19; mediana 0,84%) (p≤0,05). **Conclusión:** las IR propias permiten desvelar las condiciones de salud de los adultos brasileños y pueden subsidiar la correcta identificación de la enfermedad renal crónica y la diabetes.*

Palabras clave: *Valores de Referencia; Creatinina; Hemoglobina A Glucada; Encuestas Epidemiológicas; Factores de Riesgo; Brasil.*

INTRODUCTION

The Chronic Kidney Disease (CKD) and diabetes diagnoses depend on the selection and monitoring of biochemical markers1 such as creatinine - which is relevant for estimating Glomerular Filtration Rate (GFR) and assessing renal function - and glycated hemoglobin (HbA1c) - important for assessing glycemic control.[2,3] Thus, obtaining accurate Reference Intervals (RIs) of these laboratory tests is fundamental in the clinical practice for the diagnosis, prevention and treatment of these diseases.[4]

RIs are one of the most important elements of laboratory tests, as they are used for the interpretation of results, being essential information for decision-making.[4] To ensure proper interpretation of test results, RIs determined based on a healthy population are necessary.[1,5] However, the determination of RIs represents a challenge for health systems, due to the need to obtain a representative sample of the population,1 as well as care in collection, transportation, biochemical analysis and statistics.[4] In addition to that, there is diverse evidence that biological, environmental, cultural and individual factors can influence the RIs of laboratory tests, such as race, ethnicity,[6] circadian rhythm, diet, pregnancy, menstrual cycle,[6,7] menopause,[6] practice of physical activity, stress, smoking, medication use, and consumption of alcoholic beverages or caffeine[7].

The usefulness of creatinine for the identification of renal failure is complex due to its variation according to gender, age, race, diet and muscle mass of the individual in which it is being measured.[2] HbA1c differs across racial groups; therefore, adopting the standard RI may not be useful in some populations.[3] Therefore, for a reliable interpretation, the ideal is to determine RIs appropriate for the population in which they will be applied.[8]

In Brazil, for the adult population, RIs from the international literature[7] and from other countries4 are used. However, in view of miscegenation and epidemiological and cultural aspects that distinguish the Brazilian population from other countries, it is important to obtain appropriate RIs so that they are adequate.[4] To the present, only one study has been carried out in Brazil, with laboratory data from the National Health Survey (PNS), in which reference values for creatinine and HbA1c were estimated in adults using the parametric method.[4] Therefore, it becomes necessary to carry out other studies applying different methods of RI determination, as the literature documents that using a single approach can lead to imprecision, with indication of testing other methodologies.[9]

In addition, it should be noted that laboratory data from the PNS identified, in Brazilian adults and in 2014-2015, that the prevalence of CKD was 6.5%,[10.11] and that the one for diabetes varied between 6.6% and 9.5%.10 Therefore, given these prevalence values, for proper management of these diseases in the country in the clinical practice, it is important to know appropriate RIs considered as normal.[10.11]

This study innovates by establishing creatinine and HbA1c RIs by means of a method not yet used, the non-parametric method, taking into account the recommendations set forth in Guideline C28-A3,[12] which is the most widely used source in the laboratory area.[5] Another novelty is the expansion of the exclusion criteria adopted in the national study by Szwarcwald et al.,[4] to get closer to the healthy population. This study contributes to the improvement of RI calculation methodologies for the Brazilian adult population, in order to obtain increasingly reliable values.

In this sense, the objective was to estimate creatinine and HbA1c RIs for the Brazilian adult population.

METHOD

Study design

A cross-sectional study with laboratory data from the PNS carried out between 2014 and 2015.

Context

The PNS is home-based and is carried out by the Brazilian Institute of Geography and Statistics (IBGE), the Ministry of Health and the Oswaldo Cruz Foundation.[13,14] The target population consisted of people living in permanent private households and belonging to the census tracts of the 2010 Geographical Operational Base.[13,14]

Due to the PNS complex sampling design and the unequal selection probabilities, sample weights were used.[13,14] The 2013 PNS was conducted in 64,348 households and

interviewed 60,202 adults.[13,14] For the selected adult resident, weight, height, waist circumference and blood pressure measurements were taken; in addition, in a subsample of 25% of the census tracts surveyed, blood was collected for laboratory tests in 8,952 individuals.[13,14]

The laboratory test collections were performed at any time of the day, without fasting, and were analyzed using an automatic cell analyzer. HbA1c was collected in a tube with ethylenediamine tetraacetic acid and dosed by ion exchange high-performance liquid chromatography. Creatinine was collected in a gel tube and measured by means of the Jaffé method, without deproteinization.[4]

Data source

The data used are freely accessible and were obtained from the PNS repository: https://www.pns.icict.fiocruz.br/.

Participants

In this study, the subsample of the PNS laboratory tests consisting of 8,952 adults aged 18 years or older was used.

Determining the reference intervals

To reduce the factors that can influence the RIs and reach a population considered healthy, exclusion criteria were applied according to the literature.[1,4,8,12] The exclusion criteria were as follows: age < 18 years old; obesity (Body Mass Index ≥ 30 kg/m^2,[15] calculated by weight and height measurements); arterial hypertension (self-reported and measured blood pressure ≥ 140/90 mmHg);[16] CKD (GFR < 60 mL/min/1.73m^2, calculated by means of the Chronic Kidney Disease Epidemiology Collaboration equation);[17] diabetes (self-reported and HbA1c ≥ 6.5%);[18] hemoglobinopathies (sickle cell trait, persistence of fetal hemoglobin, sickle cell trait with hemoglobin C, minor thalassemia, suspicion of major and minor thalassemia and carriers of hemoglobin C) and anemia (hemoglobin: women < 12 and men < 13 g/dL[19]); adults without test results or who presented pregnancy, smoking habit, chronic obstructive pulmonary disease, cardiovascular disease, stroke, arthritis or rheumatism.

Outlier exclusion was performed by visual inspection and by means of the Tukey method, which defines upper limit (UL) and lower limit (LL) by the interquartile range (IQR), first quartile (Q1) and third quartile (Q3) (LL: Q1-(1xIQR); UL: Q3+(1xIQR). Data outside these limits were considered outliers.[5]

The sample was partitioned by gender, age and race/skin color. The need for stratification was verified by the existence of statistical differences between the groups and by the biological conditions that influence the RIs.[6]

The RIs were determined considering 95% of the healthy individuals,5 linked to the 2.5th and 97.5th percentiles,12 Samples of more than 120 individuals were used in the subgroups partitioned by gender and age.[12]

Os IR foram determinados considerando 95% dos indivíduos saudáveis,[5] ligados aos percentis 2,5 e 97,5,[12]. Utilizaram-se amostras acima de 120 indivíduos nos subgrupos particionados por sexo e idade.[12]

Variables

The included variables were the following:

Sociodemographic: gender (male; female); age (age group in years old: 18-29; 30-44; 45-59; 60 or more); and race/skin color (white; brown; black).

Creatinine and HbA1c: creatinine collected value (mg/dL) – variable code Z025; and glycated hemoglobin collected % value – variable code Z034.

Statistical analyses

The medians of each partition and the reference limits were calculated, with the LL linked to the 2.5th percentile, and the UL to the 97.5th percentile of the distribution of the reference population, according to gender, age and race/skin color. Data normality was evaluated by means of the Shapiro Wilk test.

To determine the RIs, the non-parametric method was adopted, which sorts the observations made by size and classifies them considering from the lowest r = 1 to the highest r = n. The LL corresponded to r = 0.025 (n+1,) and the UL to the observation of position r = 0.975 (n+1) of the ranking[12].

The differences were evaluated by means of the *Mann Whitney or Kruskal Wallis tests*, with Dun's post-test and *Bonferroni's* correction. The significance level adopted was 5%.

The analyses were performed in the *Data Analysis and Statistical* (Stata) software, version 14, and in the *Package for the Social Science* (SPSS) software, version 25.0, using the *survey* module, which considers the post-stratification weights.

Ethical aspects

The PNS was approved by the National Research Ethics Committee of the National Health Council, Ministry of Health, under number 328,159. Participation of the adults was voluntary and confidentiality of information was ensured.[14]

RESULTS

The laboratory PNS consisted of 8,952 adults. Due to insufficient material, hemolysis and sample loss, 411 test samples were excluded for HbA1c (with 8,541 adults remaining at this stage) and 417 samples for creatinine (with 8,535 adults remaining at this stage). According to the exclusion criteria established, 5,739 participants were excluded from the HbA1c analyses, with 2,802 adults remaining at this stage. In the creatinine analyses, 5,733 participants were excluded, with 2,802 adults remaining at this stage. Subsequently, 79 and 64 outliers were removed in the HbA1c and creatinine analyses, respectively. The sample of this study included RIs of 2,723 adults for HbA1c and 2,738 adults for creatinine.

Table 1 presents the creatinine and HbA1c RIs according to gender. For creatinine (mg/dL), men (0.69-1.25; median: 0.95) had higher RIs than women (0.53-1.05; median: 0.74) ($p \leq 0.05$). For HbA1c (%), a small difference is perceived between the genders, verified by the higher RI and median values in males (4.55-5.97; median: 5.23) when compared to females (4.49-5.97; median: 5.20) ($p \leq 0.05$).

Table 2 presents the creatinine and HbA1c RIs according to age. For creatinine (mg/dL), women aged between 18 and 29 years old (0.52-1.04; median: 0.73) and between 30 and 44 years old (0.53-1.06; median: 0.72) had lower RIs when compared to women aged between 45 and 59 years old (0.55-1.04; median: 0.77) and from 60 years old (0.54-0.98; median: 0.77), as noted by the LL and median values ($p \leq 0.05$). In men, the creatinine RIs were similar according to age ($p > 0.05$).

For HbA1c (%), it is perceived that higher LL and UL values were recorded in men aged 60 years old when compared to younger age groups (18-29 years old: 4.53-5.90; median: 5.15; 30 - 44 years old: 4.62-5.92; median: 5.21; 30 - 59 years old: 4.52-6.00; median: 5.30; 60 years old or more: 4.65-6.07; median: 5.44; $p \leq 0.05$). Women from 45 years of age had higher RIs when compared to younger women (18-29 years old: 4.51-5.79; median: 5.12%; 30-44 years old: 4.42-5.87; median: 5.10; 30 - 59 years old: 4.61-6.05; median: 5.40; 60 years old or more: 4.82-6.03; median: 5.50; $p \leq 0.05$) (Table 2).

Table 3 presents the creatinine and HbA1c RIs according to race/skin color. For creatinine (%), higher RI values and more prominent medians were observed in white-skinned adults (0.56-1.19; median: 0.85) when compared to brown-skinned ones (0.55-1.19; median: 0.84) ($p \leq 0.05$). There were no differences for HbA1c RI according to race/skin color, as well as when stratified by both genders for creatinine and HbA1c ($p > 0.05$).

DISCUSSION

In this study, the results showed differences for the creatinine RI, being higher in Brazilian men than in women, lower in women aged between 18 and 29 and 30 and 44 years old than in those between 45 and 59 and 60 years or more, and higher LLs in white than in brown race/skin color. For HbA1c, men presented higher LL values when compared to women and men from 60 years old, and women aged between 45 and 59 years old and 60 years old or more had higher RIs than those from 18 to 44 years old. The RIs were determined in a representative sample of Brazilian adults, following

Table 1 - Creatinine and glycated hemoglobin reference intervals in adults ≥ 18 years old according to gender, National Health Survey, Brazil, 2014-2015

Exams	Gender	n	Median	Min-Max	LL	UL	p*
Creatinine (mg/dL)	Total	2,738	0.85	0.30-1.32	0.56	1.19	<0.01
	Male	1,278	0.95	0.40-1.32	0.69	1.25	
	Female	1,460	0.74	0.39-1.24	0.53	1.05	
Glycated Hemoglobin (%)	Total	2,723	5.22	4.30-6.17	4.52	5.97	<0,01
	Male	1,275	5.23	4.30 - 6.17	4.55	5.97	
	Female	1,448	5.20	4.30-6.16	4.49	5.97	

n: Sample. Min-Max: Minimum and maximum values. LL: Lower limit (2.5th percentile). UL: Upper limit (97.5th percentile). **Mann Whitney test*.

Table 2 - Creatinine and glycated hemoglobin reference intervals in adults ≥ 18 years old according to gender, National Health Survey, Brazil, 2014-2015

Exams	Age	n	Median	Min-Max	LL	UL	p*
Male gender							
Creatinine (mg/dL)	18-29	280	0.96	0.52-1.33	0.66	1.25	0,2242
	30-44	513	0.94	0.40-1.32	0.69	1.22	
	45-59	332	0.97	0.59-1.32	0.71	1.28	
	60+	153	0.97	0.69-1.30	0.72	1.25	
Glycated Hemoglobin (%)	18-29[a]	280	5.15	4.32-6.16	4.53	5.90	0,0001
	30-44[b]	521	5.21	4.35-6.15	4.62	5.92	
	45-59[c]	329	5.30	4.30-6.17	4.52	6.00	
	60+[d]	145	5.44	4.43-6.16	4.65	6.07	
Female gender							
Creatinine (mg/dL)	18-29[a]	372	0,73	0,39-1,24	0,52	1,04	0,0002
	30-44[a]	633	0,72	0,40-1,19	0,53	1,06	
	45-59[b]	322	0,77	0,39-1,10	0,55	1,04	
	60 e mais[b,c]	133	0,77	0,48-1,01	0,54	0,98	
Glycated Hemoglobin (%)	18-29[a]	368	5,12	4,37-6,03	4,51	5,79	0,0001
	30-44[a]	633	5,10	4,30-6,16	4,42	5,87	
	45-59[b]	320	5,40	4,30-6,14	4,61	6,05	
	60 e mais[c]	127	5,50	4,44-6,13	4,82	6,03	

n: Sample. Min-Max: Minimum and maximum values. LL: Lower limit (2.5th percentile). UL: Upper limit (97.5th percentile). *Kruskal Wallis test. a,b,c,d: same letters represent that there were no statistically significant differences between the groups (p>0.05); different letters represent that there were statistically significant differences between the groups (p≤0.05).

Table 3 - Creatinine and glycated hemoglobin reference intervals in adults ≥ 18 years old according to race/skin color and gender, National Health Survey, Brazil, 2014-2015

Exams	Race/ Skin color	n	Median	Min-Max	LL	UL	p*
Total							
Creatinina (mg/dL)	White[a]	1,089	0.85	0.40-1.32	0.56	1.19	0,0297
	Brownb,[c]	1,460	0.84	0.39-1.32	0.55	1.19	
	Blacka,[c]	195	0.85	0.40-1.28	0.56	1.19	
Hemoglobina Glicosilada (%)	White	1,085	5.21	4.30-6.16	4.54	6.00	0,0532
	Brown	1,400	5.21	4.30-6.17	4.50	5.96	
	Black	193	5.32	4.39-6.15	4.47	6.00	
Male gender							
Creatinina (mg/dL)	White	518	0.96	0.60-1.32	0.70	1.25	0,0619
	Brown	641	0.94	0.52-1.32	0.68	1.25	
	Black	100	0.97	0.40-1.28	0.69	1.26	
Glycated Hemoglobin (%)	White	514	5.2	4.4-6.2	4.59	6.00	0,459
	Brown	643	5.2	4.3-6.2	4.52	5.96	
	Black	100	5.3	4.5-6.2	4.67	6.00	

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Table 3 - Creatinine and glycated hemoglobin reference intervals in adults ≥ 18 years old according to race/skin color and gender, National Health Survey, Brazil, 2014-2015

Exams	Race/ Skin color	n	Median	Min-Max	LL	UL	p*
Female gender							
Creatinina (mg/dL)	White	571	0.74	0.40-1.12	0.53	1.02	0,3333
	Brown	765	0.74	0.39-1.24	0.53	1.05	
	Black	95	0.77	0.40-1.15	0.56	1.05	
Hemoglobina Glicosilada (%)	White	571	5.2	4.4-6.1	4.52	6.00	0,0665
	Brown	757	5.2	4.3-6.1	4.48	5.95	
	Black	93	5.3	4.4-6.1	4.43	5.96	

n: Sample. Min-Max: Minimum and maximum values. LL: Lower limit (2.5th percentile). UL: Upper limit (97.5th percentile). *Kruskal Wallis test. a,b,c,d: same letters represent that there were no statistically significant differences between the groups (p>0.05); different letters represent that there were statistically significant differences between the groups (p≤0.05).

recommendations from the literature, when testing the RI calculation methodology not yet applied[9] to obtain increasingly accurate and reliable values.[9] Consequently, it differed from the only existing national study,[4] when using a non-parametric approach, by means of the tests applied for sample stratification and by the *Tukey* method for outlier removal, in addition to expansion of the exclusion criteria in relation to the study by *Szwarcwald* et al.[4]

The following stages conducted in this study to obtain the RIs were based on the criteria set forth in Guideline C28-A3[12] and on population studies,[1,4,8] which consisted in applying exclusion criteria, detection of discrepant values, stratification and determination of the RI (estimated by the LL related to the 2.5th percentile and UL related to the 97.5th percentile of the distribution of the reference population).[5,12] Thus, 5% of the results of the examinations of healthy individuals who were excluded from the RIs were considered abnormal.[5] For the selection of reference individuals, it was considered that the RIs are determined based on a healthy population[1,4,8,12] and the exclusions to control influences on health when comprising the reference sample.[5,12] It is noted that, in the context of using laboratory tests, having good health is a relative condition and without a universal definition,[5] all the stages being carefully evaluated in an attempt to approach 95% of healthy individuals.[5,12]

Regarding the procedures for detection and removal of outliers, the choice of visual data inspection was made because it is considered an effective method [5] used in other studies[1,4] and the *Tukey* method for its usefulness in the presence of more than one outlier,[5] as was the case when analyzing HbA1c and creatinine in Brazilian adults. The stratification of creatinine and HbA1c RI according to gender, age and race was performed considering the physiological changes in adulthood, which can affect laboratory tests and statistical differences.[6]

In this study, after the procedures of exclusion and removal of outliers, the median values did not change for creatinine, with a slight reduction for HbA1c, with asymmetric distribution curves. The non-parametric calculation methodology is recommended by Guideline C28-A3, as many analyses do not present normal distribution 12. In the previous national study,[4] the parametric method was used, also in accordance with the literature that documents that the RI results calculated by means of these two methods are usually similar;[5] as observed by the RI values found in the national study,[4] in which the means (LL-UL) for creatinine (mg/dL) and HbA1c (%) were 1.0 (0.7-1.2) and 5.3% (4.5-6.1) in males and 0.8 (0.5-1.0) and 5.3 (4.4-6.2) in females, respectively.

Higher RIs for creatinine in the male gender were also found in studies from Brazil,[4] Canada1 and Sub-Saharan Africa,[8] indicating the influence of gender on this parameter,[8] which can be explained by the variation of creatinine with muscle mass, differing between adult men and women.[17] The differences that were found for the HbA1c RI according to gender were very subtle compared to the higher medians and LLs in males. A national study found no differences between the means, and the LLs were 4.5% in men and 4.4% in women.[4] In turn, a

study conducted with Chinese adults identified higher HbA1 levels in men than in women.[20] Possible reasons are men's greater propensity to present higher glycemic levels, which is attributed to behavioral and cultural factors of lesser health care,[20,21] evidenced by lower use of health services in Bwrazil than among women.[22]

In this study, as in the study by Szwarcwald et al.,[4] the RIs for creatinine in the male gender were higher due to higher muscle mass among men. Creatinine can be increased by physiological aging of the kidneys and by the occurrence of diabetes and hypertension with advancing age.[4]

For HbA1, the RIs were higher in older adults, as found in the national study.[4] A study conducted in China showed a positive correlation between the HbA1c levels and advancing age.[20] In Canadian adults, HbA1c showed a slight age-dependent increase in the concentrations.[23] In a research study conducted in Spain, almost half of the sample aged 65 years old or more presented average values for pre-diabetes.[24] The diabetes diagnosis is made by HbA1c > 6.5%, and values between 5.7% and 6.4% indicate pre-diabetes,[23] suggesting the presence of this last condition in Brazilian adults, whose limits exceed 5.7%, in addition to an increased risk of developing diabetes.[23] These findings can be due to the pathophysiological mechanisms of aging, such as reduced pancreatic islet function, insulin receptor activity, muscle tissue and glucose consumption.[23]

As for race/skin color, this study showed small differences for the creatinine RI between white-skinned and brown-skinned adults. In the national study, the values were similar according to race.[4] Higher serum creatinine concentrations have been documented in African descendants, and the comparability of RIs obtained in individuals from Australia, Scandinavia and Germany may reinforce that the RIs proposed are universally valid for white-skinned people.[2] However, few studies that addressed the issue of creatinine RIs in different ethnic groups meet the necessary criteria to endorse global application of RIs since, due to miscegenation, it is recommended to validate the RIs for the population in which they will be used,[12] as was done in the current study. In addition, our findings suggest the need to consider race in the definition of RIs, making it possible to define more precise diagnostic criteria that consider ethnic-racial differences in the clinical practice.

This study was limited by the possibility of including ill adults without a previous diagnosis. However, due to the representativeness of the sample, the study contributed relevant information and approached the reality of the health conditions of Brazilian adults by enabling the acquisition of values more consistent with the characteristics of the population. It should be noted that, to bring the 95% of healthy individuals closer together, the PNS database was explored and the variables [pregnant women, chronic diseases and risk factors (smoking and obesity)] that could affect the concentrations of the analyses studied were excluded.[25] This is considering the importance of having a healthy population to obtain the most accurate RIs, whose objective is to identify the clinical characteristics that are not normally found in these individuals, as well as to detect the characteristics associated with a given disease.[6] Thus, the exclusions were expanded in relation to the previous national study.4 In addition, considering that, regardless of the calculation method used to estimate the RIs, detection and exclusion of outliers are very important to obtain reliable values[5] and in order to provide greater accuracy of the results, two procedures were applied to remove discrepant values.

Although no comparison were made between the results for the RIs obtained in this research by means of the non-parametric method and the values provided by the parametric methodology used in a national study,4 as they are different methods, it is observed that the RIs of both studies were very similar, in accordance with the literature.[5] As for the losses attributed to the procedures of exclusion and removal of outliers, in the clinical practice it is a conservative bias, as it was possible to estimate the HbA1c and creatinine RIs for the Brazilian adult population herein proposed, in addition to having allowed predicting that the values found were close to those identified in the national study.[4] This is in accordance with the recommendations set forth in the literature to test different methodological approaches of RI calculations, due to the possibility of imprecision of results when applying only one methodology. Although we cannot state that one method is better than another, the RI values herein found confer greater reliability to what had already been described[4] and allows generalizing the findings of the current study to the Brazilian population in a relatively safe way.

Thus, this study shows the importance of future research studies for RI validation in Brazilian adults, contributing to a better interpretation of test results, diagnostic precision and quality of the care and treatment offered. Another limitation that should be considered is the fact that 120 individuals are recommended[12] for the partitioned strata, but this value was not obtained for women of black race/skin color. However, it is possible to determine RIs of 95% with 40 samples by means of the non-parametric method.[25] In addition, the RIs stratified by gender

according to race/skin color did not present statistical differences, showing that stratification was not necessary, but it was decided to do so respecting biological, racial and ethnic variability.[6]

CONCLUSION

It is concluded that Brazilian men have higher creatinine and HbA1c RIs than women. The HbA1c RIs increased with advancing age in both genders and, in females, this occurred for creatinine. Caucasian race/skin color adults had higher LLs than brown-skinned ones. The findings show the need for research studies to validate the creatinine and HbA1c RIs provided by means of the analytical methods proposed in Brazilian studies with PNS data, in accordance with Guideline C28-A3. In addition, the results reveal the health conditions of Brazilian adults and can support public health actions to identify and prevent diabetes and CKD, in addition to reinforcing the racial-ethnic influences on the creatinine and HbAc1 RIs.

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