BMC Nephrology

RESEARCH

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The urine protein/creatinine ratio as a reliable indicator of 24-h urine protein excretion across different levels of renal function and proteinuria: the TUNARI prospective study

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Abstract

Background The 24-h urine protein (24-hUP) excretion is the gold standard for evaluating proteinuria. This study aimed to evaluate the diagnostic efficacy of protein/creatinine ratio (PCR) for estimating 24-hUP at various levels of renal function and proteinuria levels.

Methods A cross-sectional study was conducted between December 2021 and December 2023 in Salvador, Bahia-Brazil, as an extension of previously published data from the TUNARI study. The study included 217 samples from 152 patients with various levels of renal function and proteinuria. PCR in isolated samples and 24-hUP were determined conventionally within a 24-h timeframe. Patients were classified into three groups according to the level of renal function (Group 1 = 10 to < 30 mL/min, Group 2 = 30–60 mL/min, and Group 3 = > 60 mL/min) and level of proteinuria (< 0.3 g/day, 0.3–3.5 g/day, and > 3.5 g/day). The data were analyzed using the Spearman correlation (r_s), coefficient of determination (r^2), Bland–Altman plots and receiver operating characteristic (ROC) curve. Likelihood ratios, positive (LR+), and negative (LR-) were derived from the sensitivity and specificity of PCR.

Results Mean age was 41.5 ± 15.7 years, 61.8% were women, 36.8% Black and 52% Mixed-race. Glomerulopathies constituted 80.3%; 46.1% with lupus nephritis. Of the total urine samples, we observed a high correlation between PCR in the total sample of 24-hUP sample ($r_s = 0.86$, $p < 0.001$) across different levels of renal function. However, agreement between PCR and 24-hUP was reduced at higher levels of proteinuria. The ROC analysis showed an AUC of 0.95 (95% CI = 0.92, 0.98), sensitivity of 91% and specificity of 86.5% (LR + 6.7; LR- 0.1), with an optimal cut-off of 0.77. These results were similar across renal function levels. Proteinuria ≤ 0.3 g/day showed a high sensitivity of 83.3% and specificity of 90%, with an area under (AUC) of 0.85 (95% CI = 0.71; 0.94). In the 24-hUP range > 0.3–3.5 g/day, the sensitivity was 64.1%, the specificity was 84.6%, and the AUC was 0.76 (95% CI = 0.67; 0.84), PCR detected all cases > 3.5 g/day.

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Conclusions PCR is a suitable measure to be used as an indicator of 24-hUP at different levels of renal function, but may have limitations at higher levels of proteinuria. Analysis of PCR by proteinuria level found that agreement as well as sensitivity decreases at higher levels, but it maintains good specificity and is able to identify nephrotic range proteinuria.

Keywords Proteinuria creatinine ratio, 24-h urine protein, Renal function, Nephrology

Introduction

The 24-h urine protein (24-hUP) excretion is considered the gold standard for evaluating proteinuria. It takes into account variation in urine protein concentration throughout the day. However, relying on 24-hUP has several limitations, including technical complexities, patient management and adherence issues. These issues can lead to inadequate urine collection, and consequently, unreliable test results [1]. Considering these challenges, alternative methods such as the proteinuria/creatinine ratio (PCR) are gaining attention. However, PCR's precision in patients with different levels of proteinuria or renal function remains a topic of debate, primarily related to its reliance on isolated urine samples.

The Kidney Disease: Improving Global Outcomes (KDIGO) guidelines recommend the use of PCR as a follow-up measure [2], but although PCR is an alternative method that is easy to collect, many services prefer to use the gold standard in their routine evaluation instead of PCR. Some studies showed a strong correlation between the two methods of proteinuria, but with a lack of concordance at higher proteinuria levels [3–5].

The detection of proteinuria is critical to identify patients at high risk of chronic kidney disease (CKD) progression, thus guiding treatment and preventing adverse outcomes such as initiation of renal replacement therapy and premature cardiovascular mortality. The advanced stages of CKD are associated with poorer quality of life, worse symptoms of fatigue [6, 7] functional dependence [8], and also carry a higher risk of premature mortality [7, 9, 10].

There is some evidence on the validity of PCR when compared with 24-hUP in patients with different diseases and clinical conditions [11–13]. However, the existing data are insufficient to draw definitive conclusions about the precision of the PCR in isolated samples across varying levels of renal function [14, 15]. Although PCR is commonly used to estimate 24-hUP, its application remains controversial, with some studies challenging its precision in reflecting true 24-hUP levels [16–18].

This study focuses on determining the diagnostic efficacy of using PCR in isolated urine samples to estimate 24-hUP at various levels of renal function and at different levels of proteinuria.

Methods

Study design and sample

This analysis is part of the Prospective Study of Patients with Glomerulopathies (TUNARI), conducted at "*Hospital Universitário Professor Edgard Santos*" (HUPES) in Salvador, Bahia, Brazil from December 2021 to December 2023 (Phase 1: December 2021 to May 2022; Phase 2: May 2022 to December 2023) [4]. The cross-sectional study included a convenience sample of patients who agreed to participate and were under observation at the hospital during the study period. From the sample of approximately 250 patients, 152 patients were included and a total of 217 PCRs were performed on isolated urine samples, 24-hUP and renal function samples were collected, which constituted the sample of the present study. This study includes previously obtained and published data from phase 1 of the TUNARI study. The first collection phase involved 75 patients, of which 38 provided a single sample for both urinary methods, while 37 patients provided two samples at different time periods. The second phase of collection included 77 patients, of whom 49 provided a single sample for both urinary methods and 28 provided two samples at different time periods (Fig. 1).

Data collection and definitions

With the exception of patients with a creatinine clearance < 10 mL/min, individuals undergoing renal replacement therapy, those who have received a renal transplant, and patients with suspected urinary tract infections, all adult patients seen in the glomerulopathy outpatient clinic of the HUPES who agreed to participate in the study were included. The race of the patients was classified by predefined criteria as White, Black, or Mixed Race (Pardo in Brazil, typically combining African and European ancestry) [19, 20]. The urine samples used for calculating PCR were collected randomly, without a specific time. The collection of isolated samples for urinary PCR and 24-hUP did not exceed a 24-h interval between them. All laboratory variables were measured by automated and standardized methods in HUPES. Patient assessments encompassed a comprehensive medical history, thorough physical examination, details of drug treatment, and a battery of laboratory tests.

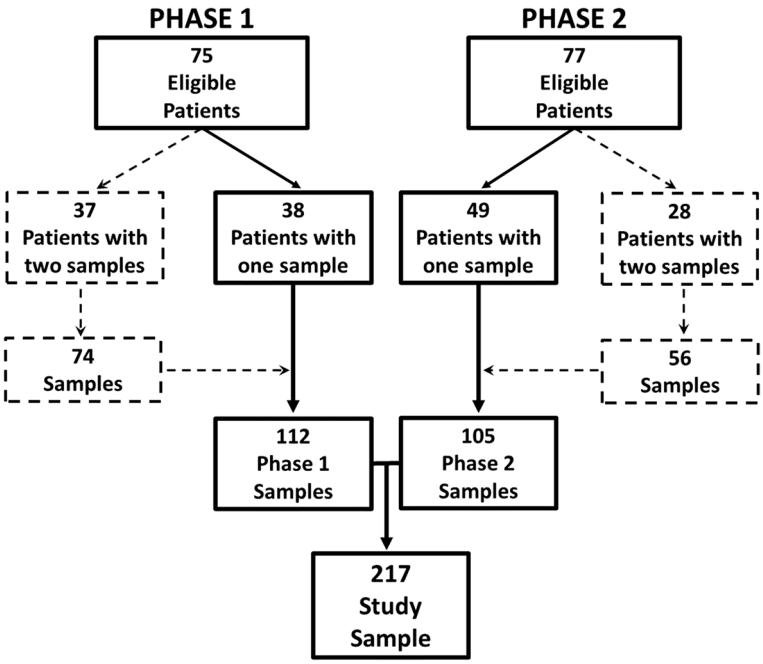

Fig. 1 Flow diagram

Assessment of patients and measurement of proteinuria and renal function

The 24-hUP samples were collected as part of routine monitoring. Samples were obtained as follows: patients were instructed to empty their bladder in the morning and discard their first urine of the day. Then, for the next 24-h, they were instructed to collect all urine in a container until the same time the bladder was initially emptied.

Clinically significant proteinuria was defined as ≥ 0.5 g/day as, according to established guidelines [3, 21, 22]. The measurement of 24-hUP and proteinuria in an isolated urine sample was performed using the benzethonium hydrochloride method. Urinary creatinine was measured by the kinetic alkaline picrate method.

The 24-h creatinine clearance was calculated using the following formula: *Clearance (mL/minute) = U/S x MV; U = urine creatinine (mg/dL); S = serum creatinine (mg/dL); MV = minute volume (24 h urinary volume, in mL, divided by 1440 min), the* creatinine clearance *was corrected for the body surface* [4].

Statistical analysis

Quantitative variables were described by mean ± standard deviation (SD), median, and interquartile range [IQR] for variables with non-normal distribution. For qualitative variables, frequency was used. Spearman's correlation coefficient (r_s), coefficient of determination (r^2) was calculated to compare the urinary PCR sample with 24-hUP, and the Bland–Altman plot was used to assess concordance. Correlation values greater than 0.75 were considered high, between 0.5 and 0.75 moderate and less than 0.5 were considered low correlations.

For the purposes of this study, a 24-hUP value of ≥ 0.5 g/day was indicative of significant proteinuria, whereas a value of < 0.5 g/day signified a not significant level. These criteria were used as the gold standard for assessing the diagnostic precision of the PCR test in g/g. The receiver operating characteristics (ROC) curve analysis was used to assess the overall diagnostic performance of PCR compared to 24-hUP value and the best cut-off point for PCR. Furthermore, using the sensitivity and specificity values derived from comparing PCR with the 24-hUP as the gold standard, we calculated the Youden index as well as the positive likelihood-ratio (LR+) and the negative likelihood-ratio (LR-). The Youden index was calculated as sensitivity (%) plus specificity (%) minus 100%. For PCR to be considered useful, the Youden index should exceed 0. A value of at least 50% for the Youden index is preferable, with a value close to 100% being ideal. LR+ was calculated as the sensitivity divided by the complement of the specificity, and LR- was calculated as the complement of the sensitivity divided by the specificity. The LR+ and the LR- should be instrumental in determining the probability of significant 24-hUP identified by PCR, with LR+ indicating the likelihood of proteinuria when the PCR is above the cut-off point (preferable a LR+ above 5), and LR- providing the probability of its

absence when the PCR is below the cut-off point (preferable a LR- below 0.2).

We stratified renal function, specifically creatinine clearance, into three groups: group 1, if 10 to < 30 mL/min, group 2, if 30 to 60 mL/min, and group 3 if > 60 mL/min. We also compared both methods of proteinuria according to the level of proteinuria (< 0.3 g/day, 0.3–3.5 g/day, and > 3.5 g/day). Additionally, we conducted a comparison of 24-hUP and PCR levels in terms of percentage concordance within specific proteinuria categories (Table 5). We performed an additional subgroup analysis (second phase sample vs. first phase), as well as compared the first urine sample of all patients with all samples (Supplementary Tables 1–2 and Supplementary Fig. 1A-B), where there were no notable changes. The inclusion of these additional samples helped us to increase the power of our analyses and did not represent a potential bias. Statistical analysis was performed with SPSS full form version 25.0 and R version 4.1.1.

Results

The study sample consisted of 152 patients, providing a total of 217 urine samples. The average age was 41.5 ± 15.7 years. Among the participants, 61.8% were female, and the racial composition was predominantly Black (36.8%) and Mixed-Race (52%). Glomerulopathies constituted 80.3% of our population, with lupus nephritis being the most prevalent glomerulopathy at 46.1%, followed by focal and segmental glomerulosclerosis at 15.8%. Non-glomerulopathies included arterial hypertension and diabetes mellitus. The frequency of biopsy-proven diagnosis of patients with glomerulopathy was 4/5. The main associated comorbidities were arterial hypertension (51.3%), diabetes mellitus (14.5%) and heart failure (8.6%). The median creatinine clearance was 60.8 [IQR = 31.1; 96.6] mL/min. Corticosteroid therapy was administered to 63% of patients, and 54% were under immunosuppressive therapy. Additionally, all patients were receiving either an angiotensin-converting enzyme (ACE) inhibitor or an angiotensin receptor blocker (ARB), as depicted in Table 1. We describe in Table 2 the main sociodemographic and laboratory characteristics stratified by levels of kidney function.

Table 2 shows that patients by renal function level (Group 1: 10 to < 30 mL/min) were, on average, older (42.4 years) than patients with better renal function level (Group 3: > 60 mL/min), 36 years. Sex distribution was consistent across all groups, with the majority being Mixed-Race (> 43.6%), followed by Black (> 35.2%). Hemoglobin levels were lower in patients with worse renal function level. Renal function parameters, such as creatinine and BUN, were significantly higher in groups with more compromised renal function. Proteinuria

Table 1 Main sociodemographic, laboratory, therapeutic and comorbidity characteristics

Variables	Total samples (%) *N* = 217
Sociodemographic, mean ± SD, (%)[a]	
Age in years	41.5 ± 15.7
Female	61.8
Race	
Black	36.8
Mixed-Race	52
White	11.2
Laboratory tests, mean ± SD[a]	
Hemoglobin, g/dL	10.5 ± 3.1
Albumin, g/dL	3.1 ± 1.1
Calcium, mg/ dL	8.6 ± 0.9
Phosphorus, mg/ dL	4.0 ± 1.2
Kidney function, median [IQR]	
Creatinine, mg/dL	1.2 [0.8; 2.2]
BUN, mg/dL	26.0 [15.4; 43.4]
Creatinine clearance, mL/min	60.8 [31.1; 96.6]
Urine Summary, median [IQR]	
24-h urine protein, g/day	2.1 [0.5; 4.0]
Protein/creatinine ratio, g/g	2.0 [0.6; 3.9]
Immunosuppressive therapy / Nephroprotection (%)[a]	
In use of prednisone	63.2
In use of immunosuppressants	54
Use of ACE-inhibitor /ARBs	100
Glomerulopathies (%)[a]	
Lupus nephritis	46.1
Membranous nephropathy	3.9
Focal Segmental Glomerulosclerosis	15.8
Minimal injuries	1.3
IgA nephropathy	1.3
ANCA-associated vasculitis	2
Rapidly proliferative glomerulonephritis	3.3
Membranoproliferative glomerulonephritis	1.3
Amyloidosis	5.3
Others[b]	19.7
Associated comorbidities (%)[a]	
Hypertension	51.3
Diabetes	14.5
Hearth failure	8.6
Cerebrovascular disease	0.7
Peripheral vascular disease	3.3
Chronic liver disease	7.9

[a] These variables correspond to results of the 152 patients

[b] Others = Nephropathies due to arterial hypertension and diabetes mellitus

SD Standard Deviation, *IQR* Interquartile Range, *BUN* Blood Urea Nitrogen, *ACE-inhibitor* Angiotensin Converting Enzyme Inhibitor, *ARBs* Angiotensin Receptor Blockers

Table 2 Sociodemographic, laboratory characteristics according to the levels of renal function

Variables	Group 1 = 10 to < 30 mL/min, *N* = 54	Group 2 = 30-60 mL/min, *N* = 55	Group 3 = > 60 mL/min, *N* = 108
Sociodemographic, mean ± SD or (%)[a]			
Age in years	42.4 ± 17.1	43.1 ± 16.2	36.0 ± 12.1
Female	64.8	65.4	70.4
Race			
Black	35.2	45.4	38.9
Mixed-Race	48.1	43.6	54.6
White	16.7	10.9	6.5
Laboratory tests, mean ± SD[a]			
Hemoglobin, g/dL	8.8 ± 2.4	11.4 ± 2.5	11.5 ± 2.5
Albumin, g/dL	3.1 ± 1.4	3.1 ± 0.8	3.1 ± 1.0
Calcium, mg/dL	8.5 ± 1.0	8.6 ± 0.8	8.6 ± 1.0
Phosphorus, mg/dL	4.5 ± 1.4	4.0 ± 0.8	3.7 ± 0.8
Kidney function, median [IQR]			
Creatinine, mg/dL[a]	3.1 [2.1; 4.1]	1.6 [1.1; 2.1]	0.8 [0.7; 1.1]
BUN, mg/dL[a]	44.8 [36.4; 66.3]	30.8 [22.4; 45.7]	16.3 [12.3; 24.0]
Urine Summary, median [IQR]			
24-h urine protein, g/day[a]	2.1 [0.7; 4.0]	1.9 [0.4; 4.0]	1.9 [0.3; 3.9]
Protein/creatinine ratio, g/g[a]	2.7 [1.2; 6.0]	1.4 [0.3; 3.4]	1.4 [0.3; 3.4]

[a] These variables correspond to results of the 152 patients

SD Standard Deviation, *IQR* Interquartile Range, *BUN* Blood Urea Nitrogen

levels and the PCR in isolated urine samples showed only slight variations between groups.

Compared with 24-hUP (≥ 0.5 g/day and < 0.5 g/day) as the gold standard, high sensitivity (91%) and specificity for isolated PCR (86.5%) were observed. The LR + was 6.7 and LR- was 0.1, with a ROC curve AUC of 0.95 (95% CI: 0.92, 0.98; $p < 0.001$) and a Youden index of 0.775 or 77.5% (sensitivity: 91% + specificity: 86.5% - 100%). We found that the relationship between PCR and 24-h global proteinuria showed a high discriminatory ability across all levels of renal function. Significant correlations were observed, with r_s greater than 0.83, robust determination coefficients, and a consistent cut-off point (> 0.76), with an area under the ROC curve greater than 0.94 in all groups. High levels of sensitivity (> 86.4%) and specificity (> 90%) were recorded, with the highest values noted in patients with renal function between 30-60 mL/min. Both the Youden index and ROC curve values indicate excellent diagnostic capability. Additionally, the respective LR values for the three levels of kidney function are provided (Table 3).

Figure 2 presents the total scatter plot of the PCR and 24-hUP (Fig. 2A), the concordance between the PCR ratio and 24-hUP using the Bland-Atman graph (Fig. 2B) and ROC curve comparing PCR with 24-hUP as gold standard (Fig. 2C). A good correlation was

Table 3 Stratified protein/creatine ratio (PCR) data vs. 24-hUP according to renal function levels in three groups to compare correlation, coefficient of determination, the respective Youden point, and ROC curve analysis and Likelihood-ratio

Variables	Total *N* = 217	R_s/ R^2	Cut-off by Youden Index	Sensitivity (%)	Specificity (%)	Area under ROC curve, SD (95%CI)	Likelihood-ratio
PCR vs 24-h global proteinuria	217 (100%)	0.86/0.45	0.77	91.0	86.5	0.95 ± 0.01 (0.92; 0.98)	LR + 6.7; LR- 0.1
Degree of kidney function							
10 to < 30 mL/min	54 (25%)	0.83/0.35	0.76	86.4	90.0	0.95 ± 0.03 (0.85; 0.98)	LR + 8.6; LR- 0.15
30-60 mL/min	55 (25%)	0.88/0.56	0.83	93.3	90.0	0.94 ± 0.04 (0.84; 0.98)	LR + 9.3; LR- 0.07
> 60 mL/min	108 (50%)	0.89/0.55	0.80	89.5	90.6	0.96 ± 0.01 (0.91; 0.99)	LR + 9.5; LR- 0.12

SD Standard Deviation, *CI* Confidence Interval, R_s Spearman's correlation coefficient, R^2 Coefficient of determination, *ROC* Receiver operating characteristic, *LR* Likelihood-Ratio

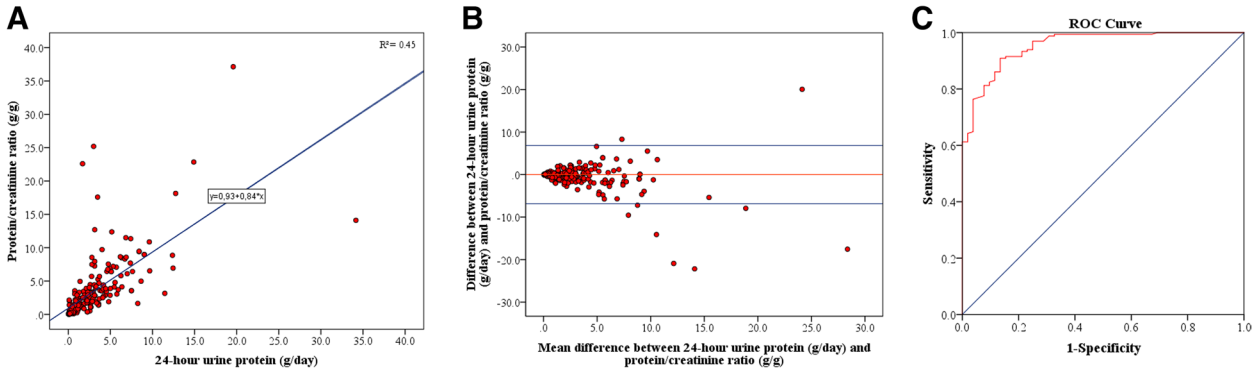

Fig. 2 **A** Total scatter plot of the protein/creatine ratio (PCR) and 24-h urine protein (24-hUP). **B** Bland–Altman graph in the evaluation of the concordance between the PCR and the 24-hUP. **C** Analysis of the receiver operator characteristic (ROC) curve (sensitivity and specificity) between the PCR with 24-hUP

observed between PCR and global 24-hUP, $r_s = 0.86$ and $r^2 = 0.45$ ($p < 0.001$). The Bland–Altman graph analysis showed a small bias (mean difference of -0.02) indicating that PCR ratio provides readings close to 24-hUP. However, there was a decrease in the concordance between PCR and 24-hUP at higher levels of proteinuria. The SD of the difference of 3.49, with an upper limit of 6.81 and lower bound of -6.85.

Figures 3, 4 and 5 presents graph analysis across three levels of renal function. Figure 3A-C presents a scatter diagram illustrating the relationship between PCR and 24-hUP, Fig. 4A-C displays a Bland–Altman plot and Fig. 5A-C shows the ROC curves for the three levels of kidney function. The findings in three graphs are very similar across levels of renal function. The Bland–Altman plot for each of the three stages of renal function

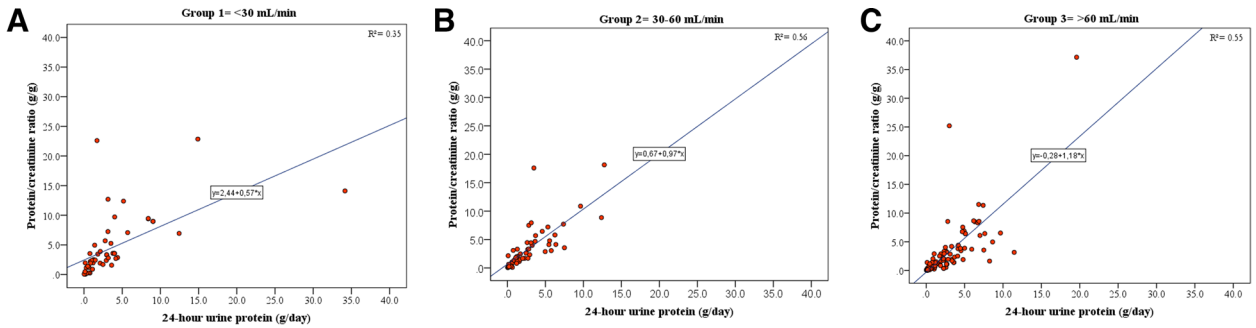

Fig. 3 Scatter plot of the protein/creatine ratio (PCR) and 24-h urine protein (24-hUP) by three levels of renal function. **A** Group 1 = 10 to < 30 mL/min. **B** Group 2 = 30–60 mL/min. **C** Group 3 = > 60 mL/min

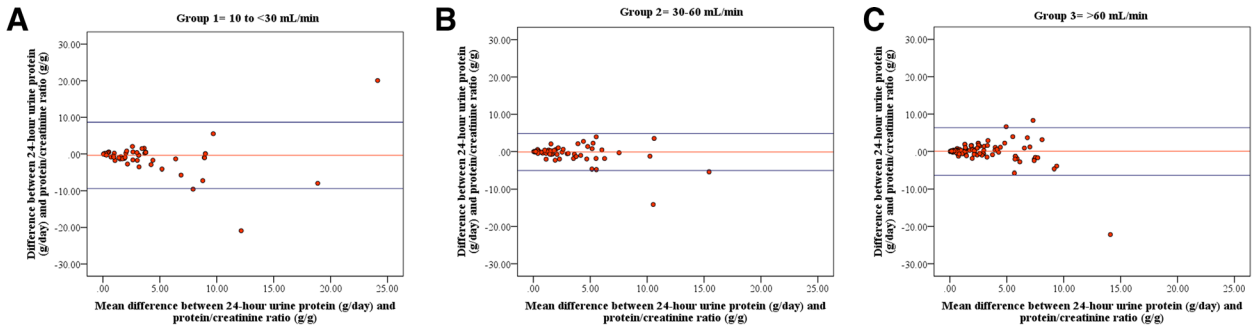

Fig. 4 Bland–Altman graph according to renal function. **A** Group 1 = 10 to < 30 mL/min. **B** Group 2 = 30–60 mL/min. **C** Group 3 = > 60 mL/min

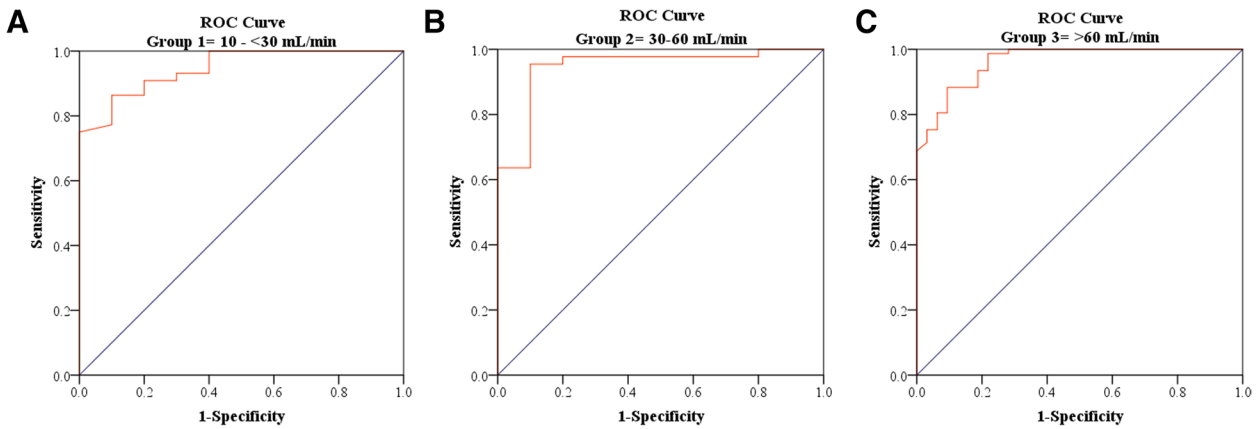

Fig. 5 Analysis of the receiver operator characteristic (ROC) curve (sensitivity and specificity) between the protein/creatine ratio (PCR) sample with 24-h urine protein (24-hUP), according to three levels of renal function. **A** Group 1 = 10 to < 30 mL/min. **B** Group 2 = 30–60 mL/min. **C** Group 3 = > 60 mL/min

indicates a reduction in agreement between methods as proteinuria levels increase across the three stages of renal function. In the ROC analysis, we observed the consistency of PCR in estimating 24-hUP across different levels of renal function, with robust performance in all groups evaluated.

Comparisons between PCR and 24-hUP, stratified by three levels of renal function, are presented in Table 3. Significant correlations were observed, with r_s greater than 0.83, robust r^2, and a consistent cut-off point according to the Youden index (> 0.76). The ROC Curve revealed a high discrimination threshold (≥ 94), supported by high sensitivity (> 86.4%) and specificity (> 90%). Additionally, the respective LR values for the three levels of kidney function are provided.

A comparison by urinary protein level is shown in Table 4. The group with proteinuria ≤ 0.3 g/day showed a sensitivity of 83.3% and a specificity of 90%, with an area under the ROC curve of 0.85 ± 0.09. The group with proteinuria between > 0.3–3.5 g/day showed a sensitivity of 64.1% and a specificity of 84.6%, and an area under the ROC curve of 0.76 ± 0.06. We observed that PCR detected 100% of proteinuria cases in patients with levels higher than 3.5 g/day, so it was not possible to calculate the ROC curve.

Table 5 shows that the percentages of agreement between 24-hUP and PCR were high across all three proteinuria categories. In particular, the agreement was 82.5% for 24-hUP levels ≤ 0.3 g/day, 74.6% for 24-hUP levels > 0.3–3.5 g/day, and 79.4% for 24-hUP levels > 3.5 g/day, for an overall percentage of 77.4% for the entire cohort. However, a decrease in the percentage of agreement is observed as the categorized proteinuria level increases, similar to that observed in the Bland–Altman analysis. This additional analysis provided a detailed assessment of how the two methods align in specific proteinuria categories. These findings outline the utility of both techniques in the evaluation of proteinuria.

Discussion

The evaluation of proteinuria in patients with CKD is a great clinical challenge, since the definition of this term can be variable, and the progression of kidney disease can be slow and asymptomatic in the early stages [14]. Measurement of 24-hUP has been an important tool in the evaluation of renal disease, but the urine samples can be inconvenient and difficult to collect. Consequently, PCR

Table 4 Comparison by degree of urine protein, correlation analysis, ROC curve (sensitivity and specificity) and Likelihood-ratio (LR)

Proteinuria Level (grams/day)	Total *N* = 217	R_s/ R^2	Cut-off by Youden Index	Sensitivity (%)	Specificity (%)	Area under ROC curve, SD (95% CI)	Likelihood-ratio
≤ 0.3 g/day	45 (21%)	0.70/0.40	0.73	83.3	90.0	0.85 ± 0.09 (0.71; 0.94)	LR + 8.1; LR- 0.2
> 0.3–3.5 g/day	105 (48.3%)	0.53/0.21	0.50	64.1	84.6	0.76 ± 0.06 (0.67; 0.84)	LR + 4.2; LR- 0.4
> 3.5 g/day	67 (30.8%)	0.36/0.20	–	–	–	–	–

SD Standard Deviation, *CI* Confidence Interval, R_s Spearman's correlation coefficient, R^2 Coefficient of determination, *ROC* Receiver operating characteristic, *LR* Likelihood-Ratio

Table 5 Comparison of proteinuria levels, 24-h urine protein (24-hUP) and protein/creatinine ratio (PCR)

Proteinuria Levels	PCR ≤0.3 g/grams	PCR >0.3–3.5 g/grams	PCR >3.5 g/grams	Total samples
24-hUP ≤0.3 g/day	33 (82.5%)	7 (17.5%)	0 (0.0%)	40 (18.4%)
24-hUP >0.3–3.5 g/day	12 (10.5%)	85 (74.6%)	17 (14.9%)	114 (52.5%)
24-hUP >3.5 g/day	0 (0.0%)	13 (20.6%)	50 (79.4%)	63 (29.1%)
Total samples	45 (20.7%)	105 (48.4%)	67 (30.8%)	217 (100%)

has been viewed as a more practical alternative for the evaluation of nephropathies. The effective identification of urinary protein excretion is essential in the diagnosis of nephropathies, as well as in the prognosis and evaluation of the therapeutic response, especially in patients with glomerulopathies. The KDIGO guidelines strongly recommended PCR, both for patients with glomerulopathies and other nephropathies [2, 3, 21].

In this study, we found a high correlation between PCR and 24-hUP in patients with different levels of kidney function, even for lower levels of kidney function (<30 mL/min). However, we observed that there was a lack of agreement between both methods at higher levels of proteinuria, which has already been described in other reports that compare the methods in different clinical conditions [4, 5, 23]. The analysis using the Bland–Altman plot revealed a loss of agreement at the most distant or extreme points on the graph. This could indicate that the measurement methods are not equally reliable or effective in all areas [23, 24]. It is important to consider these observations to better understand potential biases or inconsistencies in the comparison between methods and to make informed decisions about their use in specific situations.

The ROC curve analysis revealed a high effective of PCR to separate patients above and below a clinically significant level of 24-hUP excretion. A high sensitivity and specificity for PCR were observed both in general analysis and in subgroups of renal function. Consistent with the high sensitivity and specificity in patients across levels of renal function, a high Youden index was observed. Also, across levels of renal function, the capacity of PCR to rule in and rule out significant 24-hUP was demonstrated by the LR + above 6 and LR- below 0.15, respectively. Overall, the results indicate that PCR on isolated urine samples shows substantial discriminatory ability in patients with different levels of renal function, making it valuable for clinical decision-making [25, 26].

We used the ROC curve and AUC to compare the performance of PCR with 24-h UP, evaluating how different thresholds affect sensitivity and specificity. These metrics are key to compare the effective of both methods. With a cut-off value of 0.77 according to the Youden index, and an AUC of 0.95, along with a sensitivity of 91% and a specificity of 86.5%, these metrics are essential to correctly detect proteinuria in general. When assessed by renal function levels, the cut-off value ranged from 0.76 to 0.83 with an AUC greater than 0.94, the sensitivity was between 86.4% to 93.3% and the specificity was approximately 90%. By proteinuria levels, performance was significantly affected in the >0.3–3.5 g/day group, the cut-off point was 0.50 with a sensitivity of 64.1% and specificity of 84.6, and the AUC was 0.76. However, it is important to consider that the Youden index has limitations, such as not identifying false positives and negatives. Therefore, LR improves this evaluation by measuring how PCR results can change the probability of disease, providing a direct clinical interpretation for diagnosis.

Despite the substantial correlation between PCR and 24-hUP, and the effective measures supporting the use of PCR as a proxy for 24-hUP, the reduction in agreement at higher 24-hUP levels should be considered in clinical decision-making. For example, in the case of an acute patient with suspected lupus nephritis/nephrotic syndrome, anasarca, rapid loss of kidney function, who needs immediate action, such as immunosuppressive pulse therapy, PCR is ideal to discriminate whether the patient is with proteinuria, or not, quickly and easily. This method has high specificity, above all, it has a high capacity to discriminate whether or not the patient is in the nephrotic range (>3.5 g/day), a capacity for which it was originally designed [4, 5, 27].

In another context, where we need to follow up patients with stable conditions, treated with immunosuppression therapy and whit partial remission, we must be cautious when obtaining a result of a rapid decrease in proteinuria by the PCR method, especially when it is not accompanied by improvement of other clinical and laboratory parameters (e.g., improvement in serum albumin, improvement in renal function),

since the agreement between both methods was compromised at high levels of proteinuria [4, 5, 16, 28]. In addition, we must avoid the interspersed use between consultations of both methods, since it can show a discrepancy between them, especially in patients receiving immunotherapy when the level of proteinuria is at levels of partial remission. One should rely on the 24-hUP for patients with higher levels of proteinuria.

In the case of a stable patient, in complete remission on immunosuppressants, the use of PCR to monitor the level of proteinuria on an outpatient basis is highly recommended. In an additional analysis where we stratified the level of proteinuria, we observed a moderate correlation between PCR and 24-hUP for patients with proteinuria levels ≤ 0.3 g/day ($r_s = 0.70$ and $r^2 = 0.40$), however, the correlation was low for patients with proteinuria between > 0.3–3.5 g/day ($r_s = 0.53$ and $r^2 = 0.21$). It is imperative to highlight that the correlation was compromised in patients with proteinuria > 3.5 g/day ($r_s = 0.36$), but the effective of proteinuria detection in this group was 100% (proteinuria > 3.5 g/day), which makes ROC curve analysis unfeasible and reinforces the purpose for which PCR was created. These findings underscore the discriminatory capacity of PCR to identify patients within the nephrotic range.

Potential methodological limitations of this study should be acknowledged. One limitation is that the study was conducted within the nephrology service of a single center, which limited the sample size to the patients available in that service. The sample was restricted to the number of patients in the service and some patients who had two urine samples taken in different periods of follow-up. We performed analyses comparing the first sample of all patients to all samples as well as the subgroup assessment (phase 1 vs phase 2) and no notable changes were observed in correlation, sensitivity or specificity. The inclusion of these additional samples helped us increase the power of our analyses and markedly reduced our likelihood of bias. This study presents a majority non-White population, which reduces external validity, but is also a strong point for internal validation in non-White populations. This study was conducted in Salvador, BA, which has the largest population of African descent outside of Africa [20, 29, 30]. The Black and Mixed-Race populations have been reported to have a much higher risk of end-stage renal disease compared to the White population [31, 32]. Furthermore, studies from the United States and Brazil show that younger populations of African descent experience higher prevalence rates, worse renal survival in certain glomerulopathies, particularly focal glomerulosclerosis [33, 34], and greater mortality in the case of lupus nephritis [34, 35]. This underscores the need for heightened attention and care for these younger demographic groups, who often face worse outcomes.

The results of this study are consistent with previous studies that have demonstrated the correlation between PCR and 24-hUP in patients with different levels of renal function [14]. Furthermore, these results highlight the usefulness of the PCR as an alternative tool for the measurement of 24-hUP for the detection of proteinuria in different stages of CKD. However, caution is necessary in the use of the PCR index to substitute for 24-hUP measurements in patients with CKD and nephrotic range proteinuria, due to the observed decrease in agreement between PCR and 24-hUP at higher levels of proteinuria. In some cases, it may be necessary to measure 24-hUP to perform certain more specific assessments in patients with CKD or in patients with glomerulopathies with high disease activity, always assessing the clinical context in general. We emphasize that PCR was initially validated for the diagnosis of patients with nephrotic proteinuria and was not designed to precisely measure proteinuria [4, 5, 27].

Conclusion

The results of this study suggest that the PCR index is a practical alternative to 24-hUP measurement for identifying urinary protein in patients with nephropathies, regardless of their levels of renal function. We observed a high correlation, sensitivity and specificity, and excellent discrimination that support the use of PCR as a useful tool in the detection of urinary protein across a wide range of creatinine clearance, characterizing it as a simple and effective tool to follow the response to treatment. However, agreement between both methods is compromised at higher levels of proteinuria, requiring caution in evaluating depending on the clinical context. It is important to note that while PCR maintains good specificity and can effectively identify patients in the nephrotic range, caution is advised when interpreting PCR results from isolated urine samples. The clinical context should be carefully considered to ensure an accurate assessment.

Abbreviations

24-hUP	24-Hour urine protein
ACE	Angiotensin-converting enzyme
ARB	Angiotensin receptor blocker
AUC	Area under the curve
CKD	Chronic kidney disease
CI	Confidence interval
HUPES	Hospital Universitário Professor Edgard Santos
KDIGO	Kidney Disease: Improving Global Outcomes
LR +	Positive likelihood-ratio
LR -	Negative likelihood-ratio
PCR	Protein/creatinine ratio
ROC	Receiver operating characteristic
R	R statistical software
r_s	Spearman correlation
r^2	Coefficient of determination

SD Standard deviation
SPSS Statistical Package for the Social Sciences

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12882-024-03804-7.

Supplementary Material 1.

Authors' contributions

All authors contributed to the study conception and design. Material preparation or data collection was performed by Gabriel Brayan Gutiérrez-Peredo, Iris Montaño-Castellón, Andrea Jimena Gutiérrez-Peredo, Marcelo Lopes Barreto, Fernanda Pinheiro Martin Tapioca, Maria Gabriela Motta Guimaraes, Sony Montaño-Castellón, Samara Azevedo Guedes, Fernanda Pita Mendes da Costa, Ricardo José Costa Mattoso, José César Batista Oliveira Filho, Keith C. Norris, Antonio Raimundo Pinto de Almeida and Antonio Alberto Lopes. Data analysis was performed by Gabriel Brayan Gutiérrez-Peredo and Iris Montaño-Castellón with revisions by Antonio Alberto Lopes. The first draft of the manuscript was written by Gabriel B. Gutiérrez-Peredo and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding

This study was financed in part by the "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001."

Data availability

https://repositorio.ufba.br/

Declarations

Ethics approval and consent to participate

The Research Ethics Committee of HUPES at the Federal University of Bahia approved the study protocol, with Certificate of Presentation of Ethical Appreciation (CAAE): 64362522.7.0000.0049 and case number 5.909.208. All patients gave their informed consent to participate. The present investigation was carried out in accordance with the Declaration of Helsinki of the World Medical Association.

Consent for publication

This study has the informed consent form duly applied and signed by the participants in accordance with the ethical standards for the publication of their clinical and/or imaging data. This study included obtaining and signing informed consent from participants, in compliance with ethical standards for the publication of their clinical data and images. The anonymity and confidentiality of the information provided was strictly guaranteed.

Competing interests

The authors declare no competing interests.

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Received: 9 June 2024 Accepted: 7 October 2024
Published online: 21 November 2024

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