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Quantifying Protein loss in patients living with diabetes mellitus (PLWD) in resource-limited settings: Urine ACR or PCR?

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Abstract
Background: Nephropathy is a common morbidity associated with diabetes mellitus (DM). Proteinuria indicates renal injury which increases patients' cardiovascular risk. Presently, the recommended method for detecting proteinuria is the urine albumin-creatinine-ratio (ACR); however, this test is more exorbitant than both the urine dipstick and the protein-creatinine-ratio (PCR).
Objective: To describe the extend of proteinuria in PLWD and determine the best method of detecting it.
Method: A prospective study was performed using patients attending the diabetes clinic at Edendale Hospital between December 2017 and February 2018. Urine protein was analyzed using regression analysis.
Results: A total of 156 patients were used for analysis. Urine dipstick detected only 30 (19.23%) patients with proteinuria. The urine ACR and PCR detected 73 (46.79%) and 95 (60.9%) patients with proteinuria, respectively (p=0.09). Of these 73 and 95 patients, only 22 and 24 patients had proteinuria detected on dipstick, respectively. The positive predictive value of urine dipsticks in detecting proteinuria compared to urine ACR and PCR was 30.1% and 25.3%, respectively. The urine PCR and ACR values showed good correlation (p<0.01) on the regression analysis. A further analysis of the early renal injury protein loss category showed good correlation (p<0.01) between urine ACR and PCR.
Conclusion: There is significant protein loss in PLWD. Urine dipsticks detect early protein loss poorly. Our findings support urine PCR to detect protein loss in PLWD. This may be considered as a new primary screening modality in resource-limited settings as it provides a more cost-effective modality.

Keywords: Diabetic nephropathy, proteinuria, ACR, PCR, urine dipsticks

Introduction
Diabetes mellitus (DM) is a chronic, metabolic disease characterized by elevated levels of blood glucose, which over time can lead to damage to the heart, blood vessels, eyes, kidneys and nerves [1]. In Africa, approximately 60% of adults living with DM remain undiagnosed [2]. Estimates from the 2019 International Diabetes Federation (IDF) suggest that by 2045 there will be 700 million PLWD globally [3]. Target blood glucose levels are not being achieved at public sector diabetes clinics in South Africa, with similar results also being reflected in the United Kingdom [4, 5]. Poor glycaemic control translates to an increased burden of both micro- and macro-vascular complications [6].
Diabetic nephropathy, a manifestation of diabetic microvascular disease, is the leading cause of chronic kidney disease (CKD) and end stage renal disease (ESRD), and is present in approximately 20-30% of PLWD [7]. The review by Noubiap *et al*. estimates an incidence of between 11 and 83.7% for CKD in PLWD within African countries, depending on the method used to classify diabetic nephropathy [8]. This risk is increased in the diabetic African population, in part, possibly related to suboptimal diabetes control and elevated blood pressure from limitation of access to care [9]. The stated incidence of 20-30% of PLWD (both type 1 and 2) developing nephropathy increases as the duration of disease increases [10]. In South Africa, 50% of all causes of mortality in patients living with type 1 DM is related to renal failure [11].

This highlights the need for early and prompt therapeutic intervention, to reduce the burden of DM-related CKD on both patients and healthcare services. Angiotensin converting enzyme (ACE) inhibitors have been shown to reduce proteinuria more effectively than other anti-hypertensive medication [12]. It has also been shown to prevent renal function deterioration [12].

Proteinuria is a cardinal sign of kidney disease [13]. In PLWD with duration of diabetes between 5-10 years, 32-57% have microalbuminuria [11]. Proteinuria is a marker of renal injury and is often detected earlier than a decline in glomerular filtration rate (GFR) [14]. In addition to this, it also serves as an independent risk factor for cardiovascular morbidity and mortality [14]. Screening by means of collection of urine over a 24-hour period remains the gold standard [15]. The inherent problem of this method, especially in the resource limited setting, is that it is cumbersome test. Thus, it has been largely replaced by either urine protein reagent strips (dipsticks) or spot urine collection for albumin-to-creatinine ratio (ACR) or spot urine-protein-to-creatinine ratio (PCR) testing [16]. In our referral clinics, urine dipsticks serve as the basis for screening. The evidence largely demonstrates that this a poor screening test as the test can miss microalbuminuria [17] and hence delay the initiation of anti-proteinuric therapy. This has opened up the discussion as to whether ACR is better than PCR for screening purposes [18]. The South African diabetes guidelines advocates for ACR as the preferred test for proteinuria as it provides the earliest marker of glomerular disease [19]. In South Africa, the cost of ACR is almost three times the cost of the PCR test, the price of each test in the public healthcare sector is R80.05 vs R28.45, respectively (p<0.001)20 [20]; while the cost of a urine dipstick test is under R2,00 and thus is the favoured screening test.

Zamanzad *et al*. commented on the shortfalls of using urine dipsticks for assessing for proteinuria [21]. They found that the sensitivity, specificity, positive and negative predictive values of the dipstick test for detection of protein were 80.0%, 95.0%, 22.2% and 99.6% respectively [21] while Yadav *et al*. found that the sensitivity and specificity of the urine PCR to detect significant proteinuria at the cutoff of 0.15 are 96.6% and 74.4% respectively [22]. This illustrates that urine dipsticks have a poorer sensitivity in diagnosing proteinuria, resulting in more missed cases and less intervention to prevent the development of renal disease.

The Kidney Disease Improving Global Outcomes (KDIGO) 2012 guidelines classify CKD based on glomerular filtration rate and albuminuria. Detection of albuminuria is not always accessible or available, and for this reason, allowance has been made for severity to be determined using urine PCR and urine dipsticks [23]. The National Institute for Health and Care Excellence (NICE) 2014, Society for Endocrinology, Metabolism and Diabetes of South Africa (SEMDSA) 2017, and the KDIGO 2012 guidelines all recommend urinary ACR as the preferred tool versus PCR. KDIGO also allows for urine PCR and dipstick, however, they are still considered to be inferior to urine ACR [23].

The aim of our study was to determine if urine dipstick or PCR could replace urine ACR in detecting proteinuria in a resource limited environment.

Methods: A prospective study was undertaken at the diabetes clinic (outpatient clinic) of Edendale Hospital. The study was conducted over a three-month period (December 2017 to February 2018). One-hundred and fifty-six patients over the age of 18 years were included in the study. Both patients living with type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM) were used in this cohort. Pregnant patients or those with evidence of urinary tract infection were excluded from the study.

No control group was utilized due to the financial constraints of screening for proteinuria in low risk groups. A random cohort of PLWD was utilized as we wanted to also determine the extent of proteinuria in our sample size.

Informed consent (using consent forms translated into isiZulu language) was obtained from all patients for urine collection and testing at their clinic visit. Patients underwent standard review of bloods for their most recent serum creatinine values. The nursing staff at the diabetes clinic collected the 15ml of freshly voided urine sample. This was an early morning specimen and done by the same nursing practitioners for all the specimens. The urine was sampled using a urine Makromed® dipstick. Patients were asked to urinate into a specimen container which was brought to nursing staff who then inserted the dipstick into the urine in the containter. The urine dipstick was analysed to look for colour changes which correlated to the colour on the urine dipstick container to determine if proteinuria was present. The sample was then separated into 2 bottles and transported as 'Urgent' when taking the specimens to the lab in order to prevent laboratory errors due to delays the first was run at the NHLS for the albumin creatinine ratio (ACR). This testing was run on a Siemens® Dimension system. The second sample was sent to Global® Labs to determine the protein creatinine ratio (PCR). This testing was performed on a Synchron® System. The different tests were run at two different laboratories due to the unavailability of the PCR test at the usual testing site.

All patients who were present during our time period of the study who met our inclusion criteria were used in this study.

The study used the 2012 KDIGO classification for albuminuria and proteinuria.

Albuminuria was classified as

- <30 mg/g (normal/mild),
- 30-300 mg/g (moderate),
- or >300 mg/g (severe).

While proteinuria was classified as follows

- <150 mg/g (normal/mild),
- 150-500 mg/g (moderate),
- or >500 mg/g (severe).

And urine protein reagent strip readings were classified as

- negative or trace (normal/mild),
- trace to 1 + (moderate),
- and ≥ 1+ (severe).

The demographic data was obtained from the patient data sheet used in the clinic, and the results from both laboratories were traced using the relevant barcoded information stickers. These were captured onto a Microsoft® Excel document. Descriptive statistics (mean and standard deviation) was used to describe the sample groups. Pearson's correlation coefficient (r^2) was used to measure the statistical relationship, or association, between

two continuous variables. A p value of < 0.05 was regarded as statistically significant.

The sheet used for data collection has been approved by the University of KwaZulu-Natal's Biomedical Research and Ethics Committee (BREC) - BCA 194/5.

Ethics for this study was received from Department of Health and BREC number BE 529/17.

Patient details were kept confidential by using 'patient numbers' instead of names. After the data was made anonymous, it was analyzed for statistical significance thereafter, hence no consent was required from patients.

Results

Demographics

The results from 156 patients were used for analysis in this study (138 with T2DM and 18 with T1DM). The mean age of the sample group was 54.6 years ± standard deviation (SD) 14.89 years with a range from 19 - 90 years. Table 1 summarises the sample group findings for age, creatinine and protein loss for ACR and PCR.

Table 1: Baseline Characteristics of Study Population

	Range	Mean± SD
Age (in years)	19-90	54.6±14.89
Mean serum Creatinine (umol/l)	30-877	108±87.03
Mean spot Urine ACR (mg/g)	0.0-1005.4	37.15±111.94
Mean spot Urine PCR (mg/g)	0.0-1.269	0.08±0.18

Urine Dipstick

A total of 30 (19.23%) patients had a positive urine dipstick test for proteinuria, results of which ranged from 1+ to 4+. Urine dipstick testing missed a significant proportion of patients with proteinuria. The urine ACR and PCR detected 73 (46.79%) and 95 (60.9%) patients with proteinuria, respectively. Utilisation of the PCR method detected a greater number of patients with proteinuria versus the ACR modality (95 vs 73, p=0.09, respectively). Of these 73 and 95 patients, only 22 and 24 patients had a positive dipstick result of proteinuria, respectively. The positive predictive value of urine dipsticks in detecting proteinuria compared to urine ACR and PCR is 30.1% and 25.3%, respectively.

Tables 2 and 3 classify the total protein loss according to the KDIGO classification, into mild, moderate and severe categories.

Table 2: Quantification of Protein Loss using ACR.

Protein Loss Category	Number and Percentage of Patients
Normal or mild increase {<30 mg/g}	83 (53.2)
Moderate increase {30-300 mg/g}	40 (25.6)
Severe increase {>300mg/g}	33 (21.1)

Table 3: Quantification of Protein Loss using PCR.

Protein Loss Category	Number and Percentage of Patients
Normal or mild increase {<150 mg/g}	61 (39.1)
Moderate increase {150-500 mg/g}	48 (30.7)
Severe increase {>500 mg/g}	47 (30.1)

ACR and PCR correlation

The urine ACR and PCR were examined using Pearson's regression analysis and the r^2 value of 0.6951 showed a strong relationship for the change in ACR to correspond with PCR. This is shown in the scatterplot diagram below with a p value < 0.01 (Figure 1).

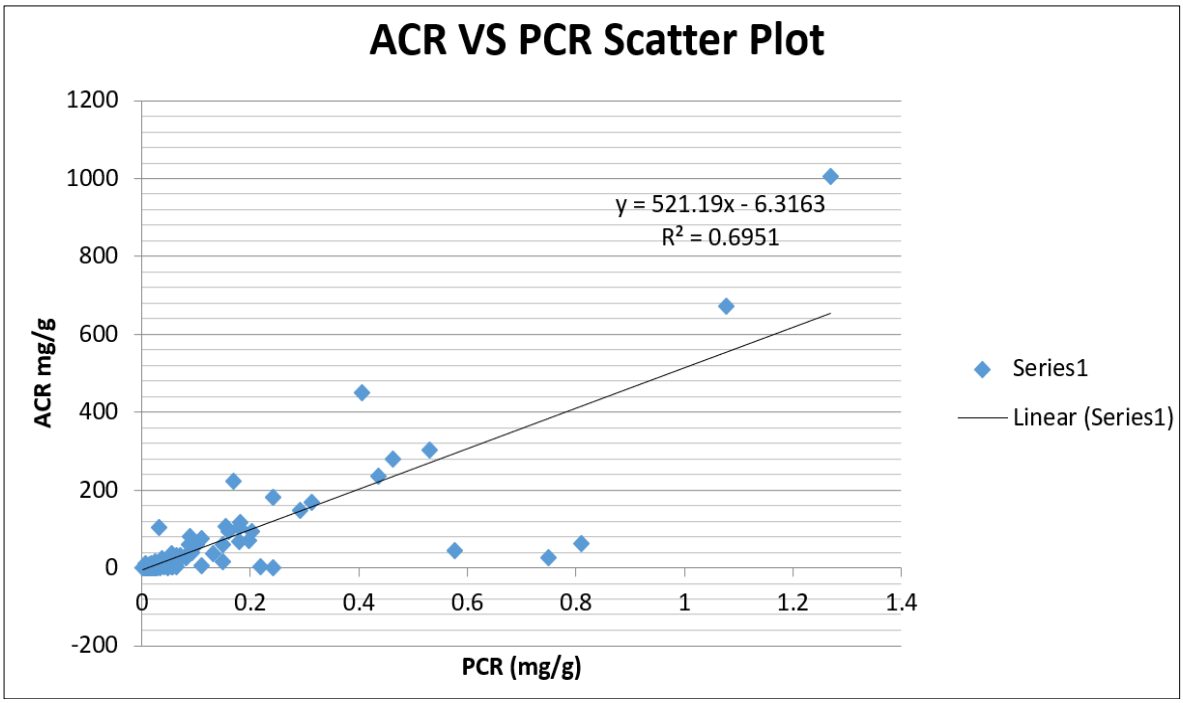

Fig 1: Correlation between urine ACR and PCR

A total of 40 ACR and 48 PCR values were noted in the early renal injury category [23]; and a further sub-analysis of 23 of these patients was done for the patients that coincided with early renal injury from the ACR and PCR group. A p-value of 0.0007 was noted, indicating a strong correlation for ACR to PCR in the early renal injury category.

Discussion

Proteinuria can be detected using various screening and diagnostic tests. Urine dipsticks are inexpensive but have been shown to be a poor screening test for CKD [25]. Additionally, at ACR levels < 30 mg/g, urine dipsticks offer low sensitivity for detection of proteinuria [26]. Collier *et al*. stated that for assessment of clinical proteinuria, either urine PCR or ACR could be used and that urine dipsticks had an acceptable sensitivity but poor specificity [27]. Our study concurred with these findings of PCR vs ACR but found urine dipsticks to offer a low sensitivity in detecting proteinuria.

It is largely agreed that urine PCR and ACR have predictive and prognostic potential, but head -to-head comparative data is scarce. There are no large studies comparing urine PCR and ACR with sensitivity and specificity outcomes in the resource limited settings, more especially looking at the diabetes population. When assessing urine PCR vs ACR, we found that urine PCR and ACR correlated well (p value<0.01) in detecting proteinuria ensuring accurate diagnosis. Similar results have been found in other studies with Fisher *et al*. concluding that ACR and PCR are comparable, and the routine measurement of PCR offered similar information as ACR in managing complications of CKD [28]. Moreover, our study found that more patients had proteinuria detected on urine PCR than on ACR. Methven *et al*. found a similar finding and concluded that PCR is a more sensitive screening test than ACR to predict clinically relevant proteinuria [29]. It is also important to note that PCR has moderate proteinuria between 150-500mg/g while ACR has moderate proteinuria of 30-300mg/g. This is important as patients with proteinuria with ACR may be more severe than those diagnosed with PCR. An example of this is for patients with proteinuria of 400mg/g - with ACR it would be severe proteinuria while with PCR it would be moderate proteinuria. Although this becomes relevant with categorical classification of proteinuria, the quantitative value of proteinuria is still able to be determined by either method and treatment can still be started at an early stage with both methods. In addition to this, it would be expected that ACR would pick up more cases than PCR for proteinuria (proteinuria should be determined once patients have proteinuria of 30mg/g in ACR compared to 150mg/g with PCR), however, this was not the case in the study.

Cost remains a major factor in a resource-limited environment. The cost of ACR is greater than PCR and has been noted that urine ACR is between 2-10 times more expensive than PCR [30]. According to the 2020-2021 NHLS pricing document [20], a saving of R51.60 can be achieved by changing the choice of test from the urine ACR to urine PCR (R80.05 vs. R28.45). According to the International Diabetes Federation, they estimate there are approximately 4 851 200 adult PLWD in South Africa [31]. The 2017 SEMDSA guidelines for management of DM recommends a yearly urine ACR as part of management [24]. If all patients followed up for screening had a urine PCR instead of a urine ACR, there would be a reduction in cost of over R250 million rand (51.60 x 4 851 200). Although unlikely that all patients will do this annual screening, a significant reduction in cost will be achieved by using the PCR over the ACR.

The good correlation with ACR and PCR in this study may serve as a start to further value its utility in resource limited settings, as it would potentially lead to cost saving in terms of testing, and ultimately, if it reduces the burden of CKD in patients with DM. The good correlation shown between PCR and ACR in this study suggests the possibility that PCR could replace ACR as the preferred screening modality in the resource limited setting, as this test can be done at referral clinics. In addition, it is important to note that the PCR is less than half the price of the ACR. This would allow cost saving, and still alert the clinician to early renal injury in the PLWD. The proven results of poor screening with urine dipsticks are re-emphasized in our study and should serve as a reminder that this is an ineffective screening tool, even in the resource limited setting.

Limitations

Patients included may have had other co-morbidities leading to proteinuria e.g. HIV-infected patients. Only one sample of urine was used for this study. No 24-hour urine sample was obtained for comparison purposes. Given the wide age range, the possibility of including
individuals who may have other conditions that may lead to proteinuria is
high. However; we are still able to determine which is the best method of detecting proteinuria despite this. Urine dipsticks contains multiple reagents that could leak into the specimen and impact results of tests.

Conclusion

Urine dipsticks have poor sensitivity in detecting proteinuria. A urine PCR and ACR have better sensitivity and correlate well. Although urine ACR is currently preferred for assessing proteinuria, our study found more patients had proteinuria detected through urine PCR than ACR. When considering the improved detection rates and the substantially cheaper cost of urine PCR than ACR, we recommend that urine PCR be considered in the screening and assessment of proteinuria.

Declaration on copyright and originality of paper

We confirm that the work is original and has not been published elsewhere, nor is it currently under consideration for publication elsewhere.

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Conflict of Interest

Not available

Financial Support

Not available

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