Proteinuria tests as useful tools in clinical practice

Complete this course and earn 1 CME POINT

Dr. HO Chung Ping
MBBS (HK), MRCP, FRCP (Glas, Edin), FHKCP, FHKAM (medicine), Specialist in Nephrology

Ms. AU Yim Fong
Manager, Integrated Dialysis Facilities

The kidneys are amongst the most obscure organs in the body. Unlike the lungs, the heart and the intestines, they do not generate any sound. Unlike the liver and the spleen, they are so deeply seated that palpation is not possible unless they are grossly enlarged. It is said (arguably) that kidneys are like the nephrologists looking after them – they work long hours quietly and diligently in the background. The easiest way to examine the kidneys is to check the substance they produce, the urine. Indeed, as a medical student, the author was taught that 'urine examination is part of a complete physical examination'.

Tests for proteinuria

It is a common belief amongst patients that frothy urine means proteinuria. While it is true that heavy proteinuria causes frothy urine, in many instances the urine froth noted by the patient is only mild and transient. It is usually due to causes other than proteinuria. The most common cause of such frothy urine is the detergent used to clean the toilet pan!

Urine froth caused by proteinuria is thick and persistent. This can be detected by placing urine in a clean test tube and shaking it (Figures 1 and 2). The urine froth will persist for more than ten minutes. This test is neither sensitive nor specific, but it is useful in bringing the patients to seek medical attention, ('the screening test of the common folks').

The commonest urine test performed in the clinics is the common urine dipstick (Figure 3). It detects mainly albumin in the urine. The test is positive when the urine albumin is >150 mg/L. It is simple and specific.

Another time-honoured test is the sulphosalicylic acid test. Protein can be detected by adding one part urine

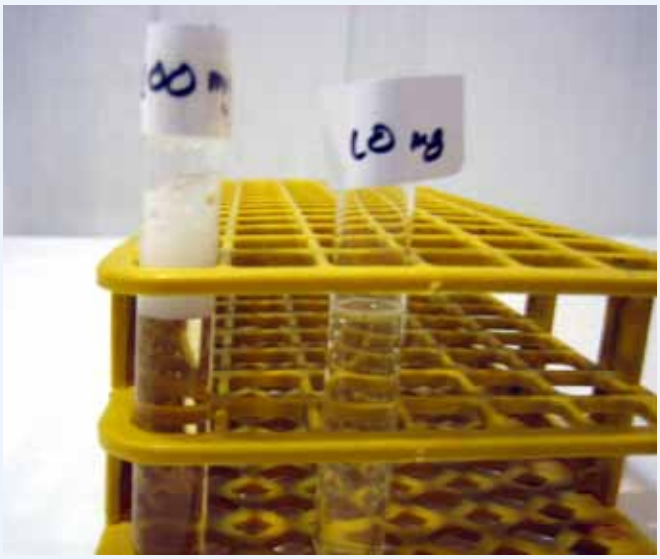

Figure 2. Urine froth lasts more than 10 minutes (left tube).

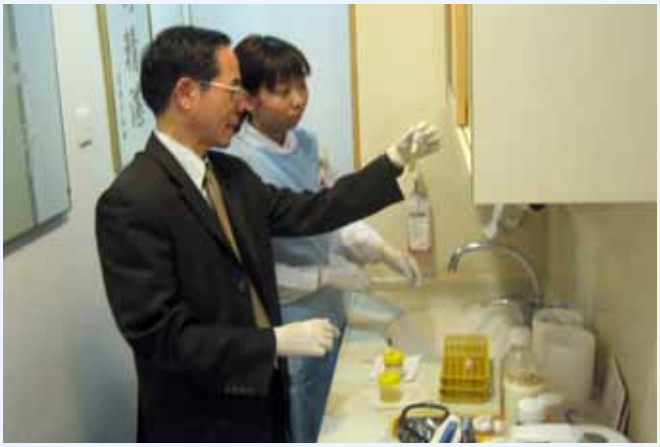

Figure 1. Shaking urine placed in a test tube.

Figure 3. Urine test with dipstick.

to three parts 3% sulphosalicylic acid and observing the turbidity (Figures 4, 5 and 6). The test can be made semi-quantitative by comparing the intensity of the turbidity (Figures 7 and 8). This test is not commonly available in most clinics. It tests both albumin and other heavier proteins in the urine. Thus the test will be positive in cases with 'tubular proteinuria' or 'myeloma kidney urine'. Since the proteins in such conditions are heavy proteins, they are not detected by the common urine dipstick.

Figure 4. Sulphosalicylic acid test.

Figure 5. Adding urine to sulphosalicylic acid.

Figure 6. Observing the turbidity.

The traditional use of proteinuria tests

The tests mentioned above are semi-quantitative only. They are useful in the management of glomerulonephropathies and nephrotic syndrome (Figure 9). Relaspes can be detected by the proteinuria tests carried out at home by the patients.

These tests only give a measure of the urine albumin (or protein) **concentration**; therefore, they will be affected by the hydration state of the patient. If a patient gives early morning urine, the urine albumin may be 100 mg/dL. After he drinks plenty of water and the same test is repeated on the new urine sample, the concentration may drop to 30 mg/dL.

For this reason, a quantitative estimation of urine protein per day is more reliable and a 24-hour urine collection is useful in this regard.

Advances made in the past two decades provided answers to the following questions:

1. Is the current dipstick urine test sensitive enough?
2. Does the quantitative urine albumin excretion have any bearing on the clinical management of renal patients?
3. Is there a less cumbersome method to measure daily urine protein excretion?
4. Are there any therapies for proteinuria?

Figure 7. Performing multiple tests.

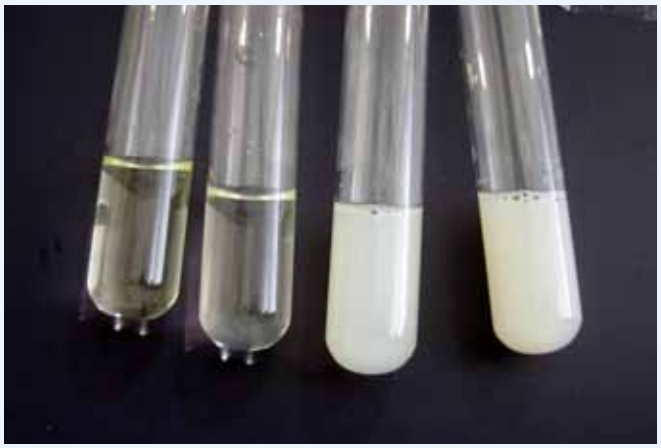

Figure 8. Different concentrations reflected by turbidity.

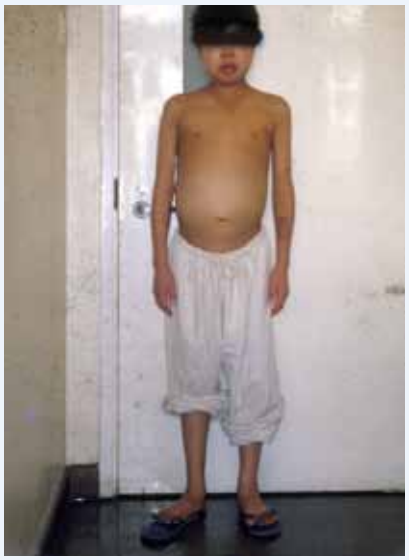

Figure 9. A patient with nephrotic syndrome.

The discovery of microalbuminuria

One of the most significant recent discoveries in nephrology is microalbuminuria in diabetic patients. It was known that in patients with incipient diabetic nephropathy, there was increased urine albumin excretion at a concentration not yet detectable by the common urine dipstick. It was called 'microalbuminuria'. The term 'micro' referred to the low urine albumin concentration and not the molecular size, as the albumin detected are normal albumin molecules [1].

In quantitative terms, normal urine contains around 20 mg/L of albumin and dipsticks can only detect around 150 mg/L of albumin. A patient is said to have **macro-albuminuria** if urine albumin reaches the concentration which can be detected by the dipstick. Albumin excretion between 20–150 mg/L is thus called **microalbuminuria**. It is an early indictor of diabetic nephropathy as the risk of diabetic nephropathy increases 10–20 times with microalbuminuria. It is amenable to treatment at this stage.

The detection of microalbuminuria required radioimmunoassay when it was first discovered and was only available in reference laboratories. With the development of immunoturbometric methods, the test is now within the reach of most laboratories. To avoid interference caused by the urine hydration, it is common to test for both the urine (micro) albumin and urine creatinine concentration and the result is expressed as a ratio. Under such circumstances, urine albumin:creatinine ratio >2.5 mg/mmol in men, >3.5 mg/mmol (30 mg/g) for women is regarded as microalbuminuria.

With improvements in medical technology and automation, the test can now be performed in renal clinics as a valuable point-of-care test. In the author's clinic, the early morning urine sample is first tested for albumin using the common urine dipstick. If the test is positive, there is macro-albuminuria and there is no need to do the expensive microalbuminuria test. If the test is negative, a screening test using the Clinitek 50 (Bayer diagnostics) machine is performed (Figure 10). It is a reagent strip test which tests both the albumin and creatinine. The colour changes are read by the machine and the urine albumin:creatinine ratio is calculated (Figure 11). The test takes one minute and is relatively cheap, but the accuracy is not too high.

If the test is positive, it is immediately confirmed with the DCA 2000 test (Figure 12), which takes 7 minutes and is considerably more expensive, but the accuracy is much higher.

Apart from incipient diabetic nephropathy, the presence of microalbuminuria also signals the increased risk of cardiovascular disease. Microalbuminuria is an inflammation marker like the C-reactive protein. It is thus a predicator of a cardiovascular event in a hypertensive patient.

Figure 10. Urine microalbuminuria screening with Clinitek 50.

Figure 11. Reagent strip with Clinitek 50 machine.

Figure 12. Urine microalbuminuria confirmation with DCA2000.

Urine albumin excretion as a clinical tool

For renal patients with macro-albuminuria, the daily urine albumin excretion correlates with the renal outcome. Patients with proteinuria <1 g/day have a much better renal prognosis than a patient with urine protein >3 g/day. Thus urine protein is of paramount importance in the prevention, diagnosis and risk stratification of renal patients.

In his excellent review article [2], Biff Palmer summarized the clinical significance of urine albumin excretion:

1. The magnitude of baseline proteinuria is an accurate predictor of the renal outcome (the renal prognosis).
2. Reduction in urinary protein excretion correlates with long-term preservation of renal function.
3. Apart from renal protection, reduction in urine albumin excretion was associated with reduction in cardiovascular risk.

From the above, it is clear that increased urine albumin excretion is a marker of poor renal prognosis. It is an indication for active interventional measures. Alternatively, reduction of proteinuria is an indication of the response to treatment and signifies improved renal prognosis.

Measurement of daily urine protein excretion

Though urine protein excretion is a useful tool, the collection of 24-hour urine is cumbersome. Recently it was suggested that we send early morning urine for estimation of the urine albumin and creatinine concentration. If both can be expressed in the same unit, (e.g. mg/L), then the urine albumin:creatinine ratio will be a 'pure' number. This number correlates closely with daily protein excretion in g/1.73 m^2 of body surface area. Thus, a ratio of 3.0 (as with respective urinary albumin and creatinine concentrations of 300 and 100 mg/dL) represents daily protein excretion of approximately 3 g/1.73 m^2. The accuracy of the total protein-to-creatinine ratio is related to the fortuitous occurrence that daily creatinine excretion is only slightly greater than 1000 mg (8.8 mmol)/day per 1.73 m^2.

Such methods avoided the timed collection of urine. It is useful for the follow-up of the same patient.

Therapeutic intervention for proteinuria

Proteinuria is a marker of renal progression. There is also evidence that proteinuria is responsible for the interstitial inflammation and fibrosis, contributing to renal progression [3]. Reduction of proteinuria is therefore of paramount importance in patient management.

From the work in diabetic nephropathy, it was shown that angiotensin converting enzyme inhibitors (ACEI) like captopril could reduce proteinuria in type 1 diabetes mellitus. Later studies showed the renoprotective effects of angiotensin receptor blockers (ARB) in type 2 diabetes mellitus patients. They were successful in increasing the time for doubling of the serum creatinine and other renal events. Apart from lowering the systemic blood pressure, the drugs dilate the efferent glomerular arterioles, thus reducing the glomerular hypertension caused by the chronic hyperglycaemia. Their anti-proteinuria effect is independent of blood pressure reduction.

ACEI and ARB were subsequently shown to reduce proteinuria in **non-diabetic** renal patients in many studies. Studies also showed that when proteinuria is reduced, the progression to end-stage renal failure (ESRD) is reduced.

ACEI or ARB can **delay** the onset of ESRD but it can be **prevented** only in a minority of cases by either drug alone. In other words, there is a limit to their reno-protective effect. A multi-tier approach is therefore needed.

For many years, there were studies researching the use of a combination of ACEI and ARB, aimed at a complete blockage of the renin-angiotensin system to achieve better renal protection. The beneficial effect was confirmed by a recent meta-analysis. It is recommended that if proteinuria is greater than 500 mg/day, despite monotherapy with an ACEI, an ARB can be added (or vice versa). Under such circumstances, monitoring of the serum creatinine and potassium is mandatory. Another approach is aldosterone blockade with spironolactone or eplerenone. There were a number of small clinical trials which suggested the benefits of add-on aldosterone antagonists, but the danger of severe hyperkalaemia is a serious concern.

Apart from lifestyle changes like stopping smoking and weight reduction, the following measures are useful:

1. Dietary protein restriction – **Moderate** dietary protein restriction would reduce the progression of renal disease. Severe dietary protein restriction (e.g. the Giordano-Giovanetti die') tends to cause protein malnutrition and muscle wasting and should be prescribed with great care.
2. Statin therapy – Some trials have shown the anti-proteinuric effect of statin therapy in early renal disease.

Conclusion

Proteinuria exists as a spectrum from microalbuminuria (>300 mg/day) to severe nephrotic range proteinuria (>3.5 g/day). Early detection of microalbuminuria in diabetic patients is the best way to prevent diabetic nephropathy. For patients with macro-albumunuria, quantitation of the proteinuria is useful in disease stratification and monitoring.

The tests for proteinuria have emerged as cost-effective and reliable ways to study the status of the kidneys and the prognosis. Three decades have passed by, and the teaching of our mentors 'urine test is part of a complete physical examination' still remain a golden principle.

References

1. Ho CP. Chronic kidney disease – old diseases with new global challenges. *HKMA CME Bulletin* April, 2008; p 4–7.
2. Palmer BF. Proteinuria as a therapeutic target in patients with chronic renal failure. *Am J Nephrol* 2007;27:287–93.
3. Ruggenenti P, et al. Kidney prevention recipes for your office practice. *Kidney International* 2005;67(Suppl 94):S136–S141.

Q&A

Answer these on page 20 or make an online submission at: www.hkmacme.org

Please indicate whether the following questions are true or false

1. The easiest way to examine the kidneys is to check the substance they produce, the urine.
2. Urine froth can be detected by placing urine in a clean test tube and leaving it to sit for one hour.
3. The common urine dipstick detects mainly albumin in the urine, and is positive when the urine albumin is >150 mg/L.
4. A quantitative estimation of urine protein per day is more reliable and a 36-hour urine collection is useful in this regard.
5. A patient is said to have **macro-albuminuria** if urine albumin reaches the 150 mg/L. Albumin excretion between 20–150 mg/L is thus called **microalbuminuria**.
6. A urine albumin:creatinine ratio >2.5 mg/mmol in men, and >3.5 mg/mmol (30 mg/g) for women is regarded as microalbuminuria.
7. A screening test for microalbuminuria uses the radio-immunoassay.
8. Apart from incipient diabetic nephropathy, the presence of microalbuminuria also signals the increased risk of cardiovascular disease.
9. A ratio of 3.0 (as with respective urinary albumin and creatinine concentrations of 300 and 100 mg/dL) represents daily protein excretion of approximately 8 g/1.73 m^2.
10. Statin therapy may be a useful intervention tool – some trials have shown the anti-proteinuric effect of statin therapy in early renal disease.

ANSWERS TO AUGUST 2009

Classification of venous disease

1. True 2. True 3. False 4. True 5. True
6. False 7. True 8. True 9. False 10. False
11. False