

A simple cost-effective screening test for microalbuminuria

H. Peiris¹, A.A.P. Keerthi¹, U.C.L. Hewage² and D. J. S. Fernando²

The Ceylon Journal of Medical Science 2003; 46: 73-81

Abstract

Estimation of urinary albumin excretion in micro quantities requires expensive immunochemical methods which are not freely available in developing countries with limited resources. A study was performed to set up a simple cost-effective test to quantitatively assess the microalbuminuria. Urine specimens (173 samples) were collected and albumin concentration was estimated by precipitating with 25% sulphosalicylic acid (SSA) and use of a light dependent resistor (LDR) system. As the sensitivity of LDR system was interfered by the colour pigment of urine, all samples were run through a Sephadex G-25 micro column to separate the colour pigment. The present test detects albumin concentrations in the range 10-200 mg/L with a sensitivity and specificity values of 83 and 79% respectively. We conclude that the present LDR micro column gel filtration method can be used as a cost-effective screening test for microalbuminuria in developing countries.

Key Words : Microalbuminuria, Sulphosalicylic Acid, Sephadex micro column gel filtration.

Introduction

Microalbuminuria has been demonstrated to indicate diabetic patients at risk of developing nephropathy. Evidence from studies show that microalbuminuria (MAU) can be reversed by appropriate clinical interventions (1,2). The commonly used definition of MAU is a concentration of 30-300 mg albumin/24 hr urine excretion which would correspond to urinary albumin excretion rate of 20-200 μ g/min (3). The development of gold standard tests such as

radio-immuno assay, immunoelectrophoresis, immunoturbidimetry methods for the measurement of low concentrations of albumin in urine made it possible to study the urinary albumin excretion in diabetic subjects before the appearance of clinical proteinuria. However, these tests are expensive, require an established laboratory and are not available in developing countries especially in the rural state sector hospitals with limited resources.

The sulphosalicylic acid (SSA) test can be performed with equipment and reagents that are available locally. The reagents for the SSA test also have the advantage of having a shelf life in excess of one year as opposed to the commercially available kits which have shorter shelf lives (4). The addition of SSA to a solution of protein results in precipitation of protein. The sensitivity of this test can be enhanced by the use of the Tyndall effect where incipient light is reflected by colloidal particles suspended in liquid. The SSA test if its validity can be proved will be the most appropriate to perform in the setting of developing countries. The objective of this study was to set up a simple, cost-effective test to quantitatively assess MAU which could be used as a screening test in developing countries.

Material and Methods

This study was approved by the Ethics Committee of the Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka.

173 urine specimens collected from 40 healthy and 133 diabetic subjects, age ranging from 30-

1. Department of Biochemistry 2. Department of Medicine, Faculty of Medical Sciences, University of Sri Jayewardenepura, Nugegoda, Sri Lanka.

54 years were used in this study. All samples were screened for microorganisms to eliminate any urinary tract infection. Urine samples from diabetic subjects were obtained from the National Diabetic Center, Rajagiriya, Sri Lanka.

Quantification of albumin in urine

The turbid urine was clarified by centrifugation and SSA test and 1 ml of clear urine was taken into a clean glass cuvette and 4 drops of 25% SSA (BDH, UK) was added. The turbidity was assessed after 3 min. under illumination from a lateral light source entering the sample at right angles to the line of vision using a portable sensitive electronic circuit system which comprise of three units, viz. Light Dependent Resistor (LDR) as a transducer, signal conditioning electronics and a display unit (Figure 1).

The LDR detects the light coming through the sample and converts it into electrical impulse and changes LDR internal resistance which is inversely proportional to the light intensity (5,6). The amount of light blocked by the sample can be detected and change of the internal resistance of the LDR is fed to the signal conditioning electronics unit. The power is supplied by 12V dry cells.

The display unit consists of LED's (Light Emitting Diodes) which have a linear scale and illuminates the corresponding LED bulb according to the input voltage given at different albumin concentrations. In order to calibrate this instrument a series of standard albumin solutions ranging from 0 to 200mg/L were prepared and held against light emitting from LDR. The maximum number of illuminated bulbs (LED's) were plotted against the standard albumin solutions (Figure 2) and such repeated measurements with different concentrations of albumin standards were used to construct a calibration scale. Using this scale one could measure the number of bulbs needed to obstruct the passage of light or the number of illuminated bulbs and thereby arrive at the albumin concentration of the constructed scale.

Sephadex G-25 superfine (Pharmacia Biotech) micro column was used to separate the coloured fraction without affecting the albumin level in urine. Six grams of Sephadex G-25 was initially allowed to be swelled in sodium phosphate buffer pH7 for 24 hours and packed in a 5ml disposable syringe. The column bed size of 1.3 x 7.5 cm. was used in the present gel filtration system as it gave the best fractionation of albumin. The flow rate was adjusted by pressing the syringe plunger to release 8 drops of urine per minute which is equivalent to 4 ml per minute. The void volume (3.5 ml) was calculated using Blue Dextran.

Urine samples (3ml volume) were run through the column bed and 1 ml fractions of first and second elution volumes were collected, pooled and precipitated with 25% SSA and subsequently albumin concentrations were estimated by the LDR method. These results were compared with the gold standard (microprotein assay kit DMA, USA cat No. 1710).

Recovery performance

Known amounts of albumin (20, 40 and 80 mg/L, DMA, USA) were added to 12 urine samples in duplicate to with known concentration of albumin and the final albumin concentrations were measured in a similar manner using Sephadex gel filtration and LDR method. The recovery rate was calculated using the initial and final albumin concentration of the samples.

Statistical Analysis

The sensitivity and specificity was calculated to assess the reliability of the test.

Results

Visual observations were made of the precipitate of protein by SSA and the turbidity was assessed 3 min. after addition of SSA (Fig. 2) using LDR method. The validity of the results by the LDR method was assessed with reference to microprotein assay method (gold standard method) by calculating the sensitivity and specificity values.

The microalbumin test (gold standard) was positive in 131 samples; of these 109 tested positive with LDR Sephadex method and gave a sensitivity of 83%. Of the 42 tested negatively by the gold standard method, 33 samples were correctly identified by the LDR Sephadex method giving a specificity of 79%. The present method detects urinary albumin concentrations ranging from 10-200mg/L.

The recovery studies indicated that the mean recovery rate and reproducibility of the results of the present method was 89 and 90% respectively (Table 2).

Discussion and Conclusions

Microalbuminuria (MAU), a marker for predicting overt clinical nephropathy is of clinical importance in both maturity onset and insulin dependent diabetes mellitus. The high albumin excretion rate in diabetes is of glomerular origin and it may be due to either abnormal permeability of glomerular membrane or increased glomerular perfusion pressure or both. The total urinary excretion in excess of 0.5g/24 hour could be detected by the conventional strip method. The development of immunoassay techniques for albumin has made it possible to detect MAU of patients who have developed proteinuria as demonstrated by the conventional tests. Unfortunately there is a lack of simple cost-effective procedures to detect albumin in urine at concentrations not detectable by conventional dipstick methods. The gold standard for albumin detection, the immunoassay method, is expensive and requires an established laboratory and is not available in government sector hospitals. The aim of the present study was to assess the validity of a simple test for screening MAU using SSA and if its validity can be proved would be the most appropriate to perform in the setting of any developing country.

The addition of SSA to a solution of protein results in precipitation of protein. The cloudy precipitate of protein was used as a bedside test

for MAU until it was superseded by the more convenient "stick" tests. Addition of 25% SSA at ambient temperature has shown no irreversible change in the conformation of albumin (7). However, temperatures above 50°C induces thermal denaturation resulting in solid precipitation of albumin.

Although the SSA test is not specific to albumin, the densitometric scanning study has shown that SSA has greater affinity towards albumin (Figure 3). In the absence of urinary tract infection, albumin could account for the positive reaction of cloudy precipitation with SSA. The results demonstrated that the turbidity reaction produced by SSA is complete in 3 min. after addition of albumin (Figure 4).

The sensitivity of estimating low concentrations of albumin has been greatly enhanced by the LDR system. The LDR is sensitive and changes its internal resistance in inverse proportion to the light intensity coming through the sample. The amount of light obstructed by the sample can be detected and this change of internal resistance of the LDR is fed to the signal conditioning electronic unit. The LDR is sensitive to detect even a faint turbidity formed by low concentrations of albumin (10mg/L) with SSA. False negative results were observed probably due to the presence of colour pigment (urochrome) in the urine. False negatives could occur due to formation of a reversible complex of albumin with urochrome or by masking the site of albumin to which SSA binds by urochrome.

The colour pigment was successfully separated by the gel filtration method (a micro column system) using Sephadex G-25 as the column material. This suggests that the bound and free form of colour pigment exists in an equilibrium state and once the free form is separated by the column chromatography the equilibrium moves towards converting more bound form to free form, resulting in more availability of free albumin for precipitation with SSA.

Albumin which has a higher molecular weight eluted out from the column immediately after the void volume. The first and second elution fractions of 1 ml carried approximately 90% of urinary albumin (Table 2). It seems that the balance 10% which is not sensitive enough to be detected by the present method may remain trapped in the column or come out with the void volume. We believe that introducing a 10% correction factor to the final value of albumin will enhance the accuracy of this method. It was found that the same column bed could be reused approximately 30-40 times after flushing out with distilled water and the approximate cost per test was Rs. 50 when compared to Rs. 350 with the gold standard (commercial microprotein assay method).

The present test detects almost all urine samples with albumin concentrations ranging from 10-200mg/L as positive with 83% sensitivity and 79% specificity. The positive predictive value of this test was 92%. Although total protein content of the urine was not measured, the present test has proven its ability to identify individuals with MAU levels from 10 - 200 mg/L. It is a reasonably safe screening method for MAU which detects nearly all patients who need further investigation, but would escape detection using the dipstick method. The fact that day to day variation in urinary albumin excretion is around 40-50% would suggest that patients with urine samples giving presumptive positive values should be investigated within short intervals of 3 to 6 month (4).

Acknowledgements

Authors wish to thank the National Science Foundation, Sri Lanka for providing a research grant to carry out this study. We are grateful to Professor E.R. Jansz, Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka for his valuable advice given in many occasions during the study. We also wish to thank Dr. K. Wanigasuriya and the staff at National Diabetic

Centre, Rajagiriya for providing specimens for this study. We gratefully acknowledge Mr. B. H. Sudhantha, Dept. of Physics, University of Sri Jayewardenepura for assisting to develop the LDR circuit.

References

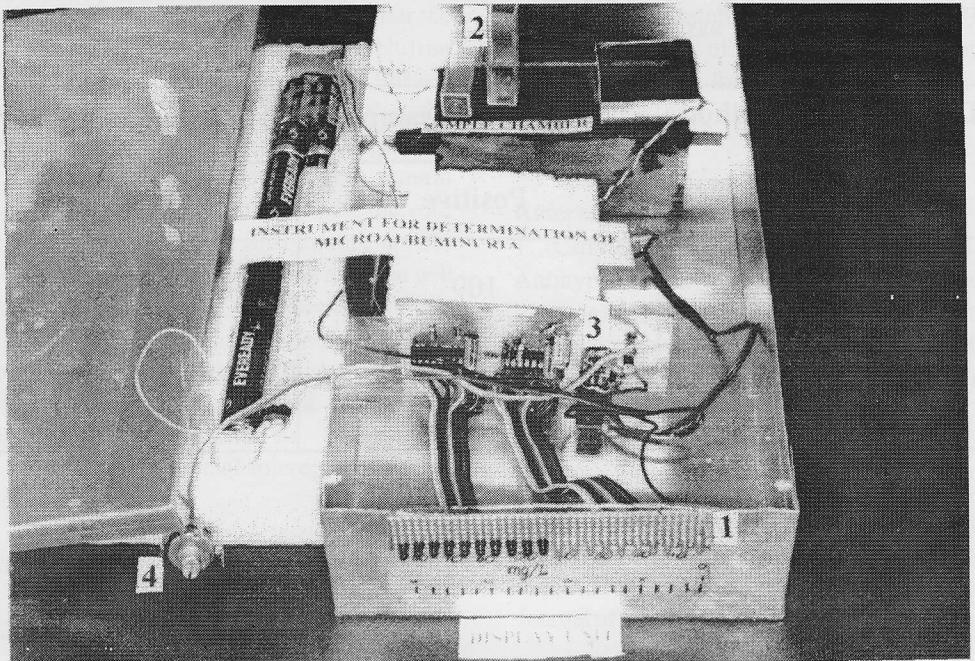
1. Christensen C.K.M, Mogensen C.E., Antihypertensive treatment: long term reversal of progression of albuminuria in incipient diabetic nephropathy. A longitudinal study of renal function. *Journal of diabetic complications*. 1987;1:45-48.
2. Viberti G.C., Pickup J.C., Jarrett R.J. *et.al*. Effect of control of blood glucose on urinary excretion of albumin and β_2 microglobulin in insulin dependent diabetes mellitus. *New England Journal of Medicine* 1985;300: 638-641.
3. Carl Erik Mogensen, William F. Keane, Peter H. Bennet *et.al*. Prevention of diabetic renal disease with special reference to microalbuminuria. *Lancet* 1995;346:1080-1084.
4. Watts, G.F., Hodgson B., Morris R.W., Shaw K.M., Polak A., Side room test to screen for microalbuminuria in diabetes mellitus. *Diabetic Medicine* 1988;6:298-303.
5. Maston R.M. *Optoelectronics circuit manual: A newnes circuits manual*. Butterworth - Heinemann, 1993.
6. Kularatne, N. *Modern component families and circuit book design*. Newnes: Butterworth - Heinemann, 2000.
7. Kaibara, K, Okazaki, T, Bohidar, H.B, Dubin, P.L, pH- Induced coactivation in complexes of bovine serum albumin and cationic polyelectrolytes. *Biomacromolecules* 2000;1:100-107.

Table 1. Comparison of LDR Sephadex method with gold standard (commercial microprotein assay method) test

LDR Sephadex Method	Number of samples with gold standard test	
	Positive	Negative
Positive	109	9
Negative	22	33
Total	131	42

Table 2 : Mean recovery rate (n=12) and reproducibility performance of LDR Sephadex method

Albumin Concentration mg/L	% Recovery rate Mean ± SD	% Reproducibility Mean ± SD
20	88.1 ± 2.8	89.4 ± 2.7
40	89.6 ± 1.8	90.3 ± 1.6
80	90.1 ± 1.2	90.7 ± 1.3



1. Calibrated display unit (LED)
2. Sample cells
3. Signal enhancing circuit
4. Controller

Figure 1. The LDR electronic circuit system

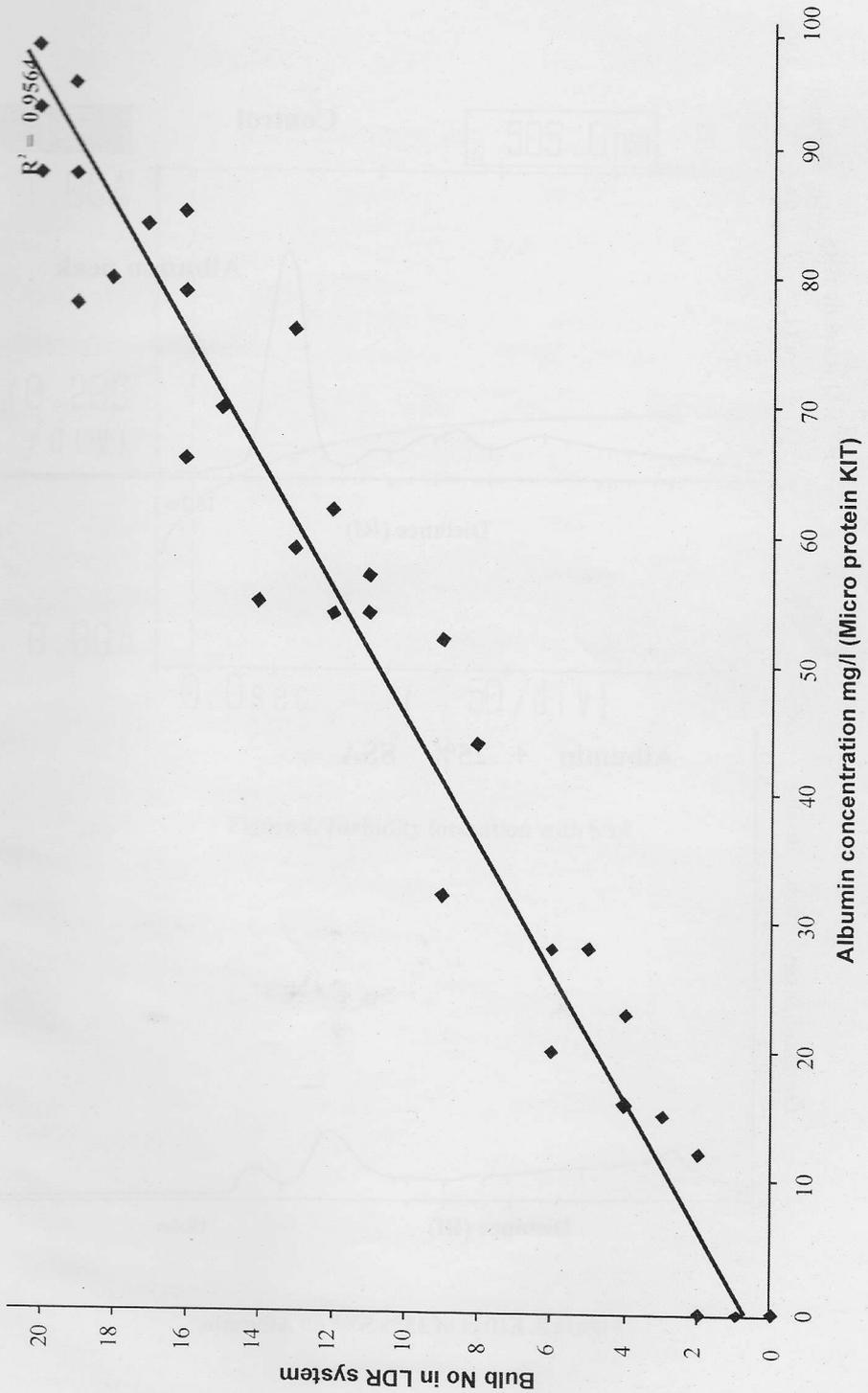


Figure 2. Comparison between LDR system and Micro protein kit

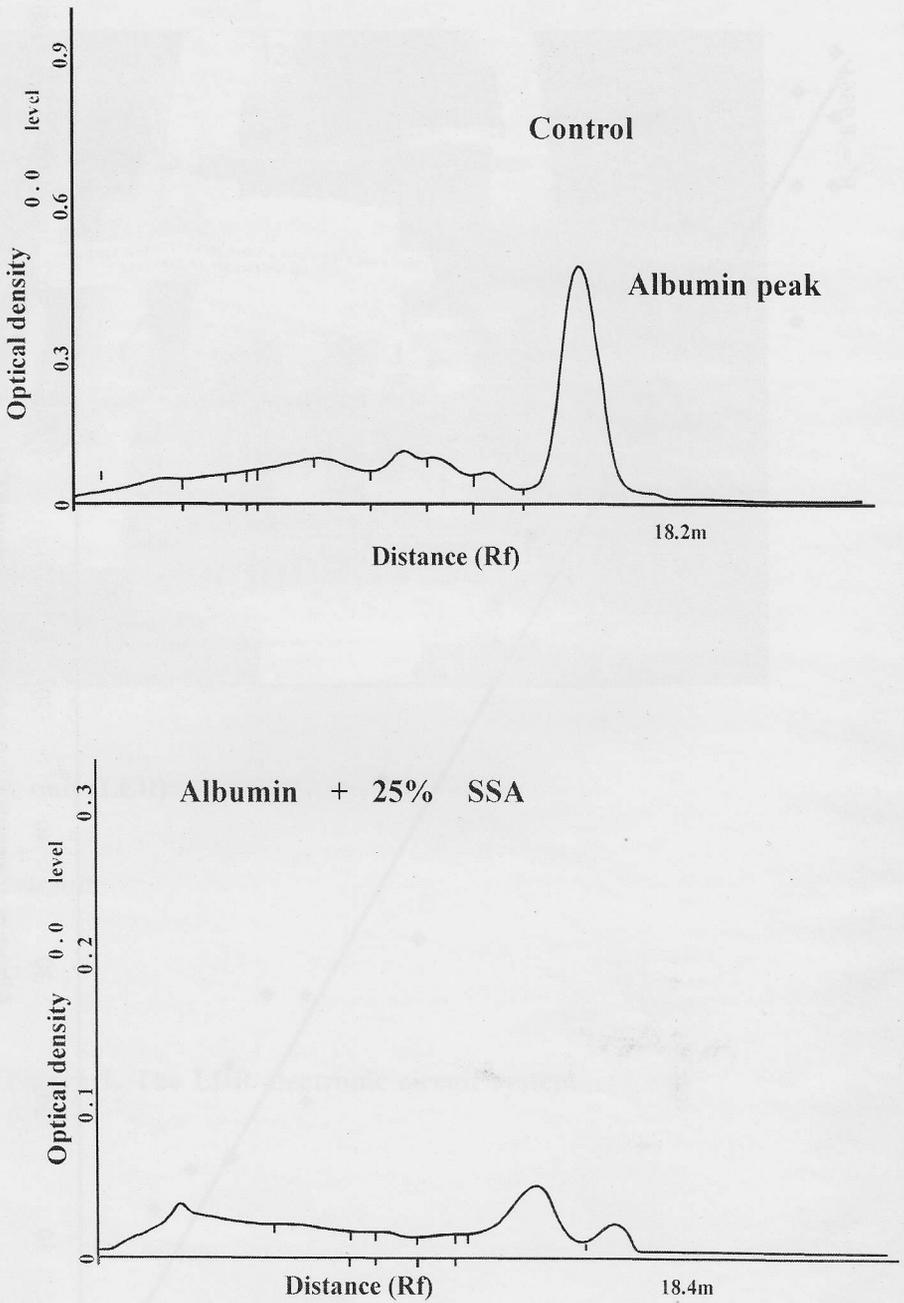


Figure 3. Effect of 25% SSA on Albumin

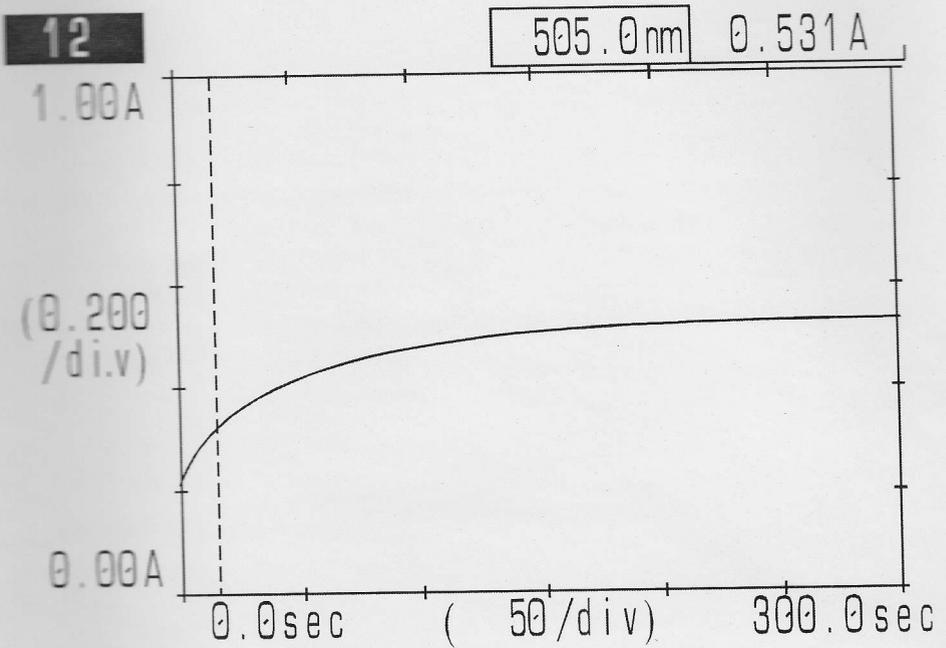


Figure 4. Turbidity formation with SSA