Student Original Article

Effect of storage temperatures and duration of storage on urinary albumin concentration in diabetics

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Abstract

Back ground and Objectives: Urine albumin measurements are used in patient care, epidemiological studies, and in clinical trials for which the samples are often stored at different temperatures and for a variable duration of time. Proper storage technique is the need of the hour to preserve the albumin concentration as measurement errors can have serious implications on the study results. Therefore, this study intended to observe the effect of storage temperatures and duration of storage on low concentrations of urinary albumin (microalbuminuria) in patients of diabetes mellitus.

Methods: Single random urine sample which is dipstick negative for protein was collected from 44 patients with diabetes mellitus. The samples were analysed immediately and then stored at -20ºC and 4ºC for 1week and 4weeks and reanalysed. The urinary albumins in the above samples were re-estimated at the end of 1 week and again at the end of 4 weeks. The difference in albumin levels between the fresh samples and after storage was noted and statistically analysed.

Results: Urinary albumin concentration decreased with the increase in duration of storage. Storage at -20°C showed a larger decrease in urinary albumin concentration as compared to those stored at 4°C.

Conclusion: Estimation of microalbumin should be performed in a fresh sample of urine and if storage is imperative, the sample may be stored at 4°C and the estimation carried out in less than a week.

Key words: Albuminuria; preservation;diabetes mellitus.

Introduction

Urine albumin measurements are used in patient care, epidemiological studies, and in clinical trials for which the samples are often stored at lower temperatures and for a variable duration of time. Practically, most of the samples are stored at -20ºC or 4ºC. Studies have shown that freezing the urine samples for storage can result in measurement errors, especially in samples with low concentrations [1,2,3]. Also, there are conflicting reports on choosing the temperature for urine storage [4,5]. Importantly, this change in urinary albumin concentrations (UACs) induced by freezing can result in a significant decrease in predictive properties of urine albumin for mortality. The variations in concentrations can also occur because of the different methods of estimations where the properties of albumin play a key role [6]. Therefore, it is necessary for every laboratory to derive its own protocol to store its urine samples depending on their method of estimation. Urine matrix components exert potentially significant effects on assays for urine albumin which varies with the population and their dietary habits [7,8]. Since there are no such studies in the Indian population, there is a need for the same. This study reports here the determinations of low albumin concentration by immunoturbidimetric method, of fresh samples, frozen aliquots (stored at -20 ºC for one and four weeks), and refrigerated samples (stored at 4 ºC for one and four weeks). Albumin was measured in 220 samples of urine obtained from 44 subjects with diabetes mellitus.

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Methods

This study was under taken in the department of Biochemistry, M S Ramaiah Medical College, Bangalore after the approval of the research and ethical committee. This study was conducted during the months of June & July, 2010. Type 2 diabetes mellitus patients attending the OPD of M S Ramaiah medical college teaching hospital were tested for urinary albumin by albustix method. Patients with gross proteinura were excluded and albustix negative diabetic patients (n=44) were recruited into the study. Turbid urine samples were also excluded from the study. Single random urine samples were collected and aliquoted into 5 polyurethane bottles. One sample was analysed afresh, 2 were frozen at -20ºC and 2 were refrigerated at 4ºC. Later the samples were reanalysed after 1 week and 4 weeks of duration of storage. The difference in albumin levels between the fresh samples and the stored samples was noted and statistically analysed. Urinary albumin was measured by immunoturbidimetric method, using fully automated analyser (ROCHE, Cobas 6000). Quantitative data was summarised to test the difference in mean values obtained for different temperatures and period of storage. A statistical analysis was done by using SPSS 14.0. Analysis of variation (ANOVA) was employed to understand the variables. Data is presented as mean (SD) & mean (SD) percentage changes in albumin. Multiple comparisons were performed with log-transformation of variables and significance was taken at 0.05 level.

Results

For analysis, results were categorised into 9 groups which are as follows:

Group 1; included the fresh sample values which acted as the controls.
Group 2; were the samples stored at 4ºC for 1 week
Group 3; samples stored at -20ºC for 1 week
Group 4; samples stored and 4ºC for 4 weeks
Group 5; samples stored at -20ºC for 4 weeks

The urinary albumin concentrations of the 5 groups were tabulated. The difference between the values in fresh samples (Group 1) and the values in stored samples i.e. Group 2, 3, 4 & 5 were tabulated as Group 6, 7, 8 & 9 respectively. Mean ± SD values of the groups are shown in the tables 1 & 2.

Table 1 shows that there is fall in urinary albumin values with storage at both 4 °C and -20°C. From table 2 it is found that storage for 1 and 4 weeks at -20°C resulted in mean (±SD) urine albumin changes of 40.78 (± 28.82) % & 62.18 (± 21.39) % respectively. Storage at 4 °C resulted in 32.14 (± 30.74) % changes after 1 week and 52.17 (± 24.77) % changes after 4 weeks. Table 3 shows the multiple comparisons of the changes after storage. It shows Group 6 is significantly different from groups 7, 8 & 9.

Table 1- Mean ± SD values of fresh and stored samples

Group	Mean ± SD (mg/L)
Group 1	17.02 ± 21.04
Group 2	11.55 ± 17.3
Group 3	10.08 ± 16.82
Group 4	8.14 ± 13.8
Group 5	6.43 ± 12.00

Table 2- The Mean± SD of amount of fall in concentration after storage

Group	Mean ± SD (mg/L)	In percent
Group 6	17.02 ± 21.04	32.14 ± 30.74
Group 7	11.55 ± 17.3	40.78 ± 28.82
Group 8	10.08 ± 16.82	52.17 ± 24.77
Group 9	8.14 ± 13.8	62.18 ± 21.39

Table 3- Multiple comparisons of the changes after storage by Log transformation

Group	Mean of the changes	Significance
Group 6 Vs Group 7	-0.0928	0.050
Group 6 Vs Group 8	-0.1604	0.001
Group 6 Vs Group 9	-0.2320	0.000
Group 7 Vs Group 8	-0.0675	0.169
Group 7 Vs Group 9	-0.1391	0.005

Discussion

The measurement of albumin in urine is important in evaluating kidney disease in people with diabetes mellitus, hypertension, or possible adverse health

effects from exposure to nephrotoxins [9]. Microalbuminuria is persistent proteinuria below level of detection by routine dipstick testing but above normal (30–300 mg/day in a 24 hour urine sample or 20-200 mg/L in a spot sample). It is an early predictor of diabetic nephropathy and also a marker of cardiovascular morbidity and mortality [10,11]. Therefore there is a great interest in accurate measurement of very low levels of urinary albumin. Epidemiological studies require that several samples be collected, stored for various periods and eventually transported to the laboratory before the assay. Hence, the temperature for storage should be appropriate so as to preserve the concentration of albumin at almost the same as that of fresh sample [12]. In this study we tried to determine the effect of storage temperatures of 4°C and -20°C when stored for 1 week and 4 weeks. It was observed that urinary albumin concentration decreased with increase in duration of storage and storage at -20°C showed larger decrease in urinary albumin concentration as compared to samples stored at 4°C, for the storage duration of both 1 week & 4 weeks. On comparing the amount of decline in albumin concentration, group 6 had the least fall (32%) and group 9 (62%) had the maximum fall in albumin concentration. Therefore, it is suggested that urine samples should be refrigerated rather than frozen and analysed at the earliest. It is thought that freezing urine specimens may cause conformational change in urinary proteins, resulting in a partial precipitation yielding low values. Precipitation of albumin at -20°C may be responsible for decreased values in this study. Also, it is found by comparing the various techniques of albumin estimation that antigen based techniques yielded low values as freezing affected the antigenic properties of albumin [13]. This study used immunoturbidimetric method which is based on the antigen–antibody reaction. Therefore, altered antigenic properties may be responsible for decrease in the albumin values at both the temperatures in this study. This false decrease could lead to classifying the albumin content as normal rather than “microalbuminuria”, a stage which can be reversed with tight glycemic control, and thus missing an opportunity for intervention. An exploration for better technique for storage is still at large. This study is limited by the fact that it did not investigate the matrix of the urine to reveal the probable factors that can cause the decline in albumin concentration.

It can be concluded that urine specimens for microalbuminuria measurements should either be analysed as fresh specimens or stored at 4ºC and assayed as soon as possible preferably within a week.

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Key Points

- Detecting low albumin concentrations in urine samples (microalbuminuria) is important for clinical management.
- Storage of urine samples for microalbuminuria determination results in reduced estimations.
- There is a greater reduction of concentration if the urine samples are stored at -20°C as compared to 4°C.
- The least decline in urine albumin concentrations is seen if the samples are estimated within a week and stored at 4°C.

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