**INTRODUCTION**

Timed urine samples (such as those collected over 24 hours) are frequently tested in clinical practice for various purposes. Important indications include assessment of the degree of proteinuria and measurement of various drug metabolites. The procedure for sample collection is undoubtedly cumbersome, but attention paid to all the relevant steps involved determines the accuracy of the results. Errors in sample collection is a well-known phenomenon. A large number of studies have been done internationally to determine the accuracy of 24 hours urine sample collections, based on urine creatinine levels. Some of these have been mentioned in the discussion to follow. However, national data on this subject is scarce. While some patients might deliberately submit inaccurate samples, others may err unintentionally. The latter is more likely to be seen in the illiterate population belonging to the rural areas. This hospital is one of the only two well established public sector setups in this region. Around 94% population of District Bagh (Azad Kashmir) and thus approximately the same proportion of the patients seen at this hospital lives in rural areas. Since such type of patients are frequently encountered at our hospital and only a very limited data on Pakistani patients is available, we carried out a study to determine the frequency of under-collected 24 hours urinary samples in type 2 diabetic patients undergoing assessment for nephropathy in outdoor clinic.

**METHODOLOGY**

This cross sectional study was carried out at the Department of Medicine, 1 Mountain Medical Battalion, Bagh, Azad Kashmir from June 2012 to November 2012 after obtaining informed written consent from the patients. As per WHO guidelines, a minimum sample size of 96 was required using anticipated population proportion of 50% (P=0.5), absolute precision of 10% (d=0.10) and 95% confidence interval. Following criteria were applied for patient selection:

- **ABSTRACT**

**OBJECTIVE:** The purpose of this cross sectional study was to determine the frequency of under-collected 24 hours urinary samples in type 2 diabetic patients undergoing assessment for nephropathy in outdoor clinic.

**METHODOLOGY:** This study was conducted on 112 patients of type 2 diabetes mellitus, who visiting outdoor clinic at the Department of Medicine, 1 Mountain Medical Battalion, Bagh, Azad Kashmir, from June 2012 to November 2012. All patients meeting the criteria were explained the procedure for collection verbally in addition to being given written instructions. Adequacy of collection was assessed by measuring creatinine excretion rates.

**RESULTS:** Out of the 112 patients enrolled, 16 (14.29%) collected their samples inadequately. Volume of inadequately collected samples was lesser as compared to samples that were collected properly (1271.25±338.68 ml vs. 1850.21±737.25 ml; p= 0.003). The education level (p<0.001) and urine volume (p= 0.003) were lesser in these 16 patients as compared to others. Inadequate collection was common in the younger patients (22.22% patients aged <60 years, 6.90% aged >60 years; p= 0.021). Gender (15.00% men, 12.50% women; p= 0.733) did not impact the collection of samples.

**CONCLUSION:** Considering the high frequency of under-collections, adequacy of samples should always be checked first before proceeding further with laboratory analysis.

**KEY WORDS:** urine collection, creatininuria, proteinuria

**Inclusion criteria:**
- Patients with type 2 diabetes mellitus, who were to otherwise undergo assessment for nephropathy in outdoor clinic

**Exclusion criteria:**
- Unwilling patients
- Patients with irregular/infrequent hospital visits (as assessed by old documents)
- Patients with impaired manual dexterity/patients dependent on others for activities of daily living

On the first visit, demographic data including education and height/body weight were recorded. All the selected patients were individually explained in detail the procedure for collecting urine samples. The collection was to start after voiding the first sample in the morning. All urine passed subsequently was to be collected in clean plastic bottles and this procedure was to be continued till the first sample voided the next morning was collected. This information was also provided in a written form to the patients. They were instructed to bring the samples to the hospital during working hours on the same day that the collection completed. On this second visit, samples were analyzed in the laboratory. Total urine volume was measured. Urinary creatinine levels were assessed using Merck Microlab 300 Automated Clinical Chemistry Analyser. 24 hours urinary creatinine excretion, calculated by multiplying these two values, was used to determine the adequacy of urine collection. Levels less than 14mg/kg/day in men and less than 11mg/kg/day in women indicated inadequate collection. Lean body mass (LBM) was estimated using the following formulas:

- For men: 
  \[ \text{LBM} = (0.32810 \times \text{weight}) + (0.33929 \times \text{height}) - 29.5336 \]
- For women: 
  \[ \text{LBM} = (0.29569 \times \text{weight}) + (0.41813 \times \text{height}) - 43.2933 \]

Data was analysed with PASW Statistics 18 using chi-Square test for determining significance of differences between various proportions and student’s t-test for differences between urine volumes. A p-value of <0.05 was considered significant.

**RESULTS**

A total of 112 patients including 80 males and 32 females were included in this study. Their clinical and laboratory profile is shown in Table 1. As depicted in Fig 1, 16 (14.29%) patients had urinary creatinine excretion below the gender specific threshold. Inadequate samples were submitted by 12 (15.00%) men and 4 (12.50%) women (p=0.733). Volume of inadequately collected samples was lesser as compared to samples that were collected properly (1271.25±338.68 ml vs. 1850.21±737.25 ml; p=0.003). Twelve (22.22%) out of 54 patients aged less than 60 years and 4 (6.90%) out of 58 patients aged 60 years or older submitted inadequate samples (p=0.021). With reference to the level of education, 14 (17.50%) out of 46 patients with up to 4 years of school education and 2 (6.25%) out of 66 patients with a greater level of education did not collect urine sample appropriately (p<0.001). Comparison of different parameters amongst the two groups (‘adequate collection of urine’ and ‘under collection of urine’) is shown in Table II.

**DISCUSSION**

Twenty four hour urine collection has over the years been regarded as gold standard for the most accurate
evaluation of urinary protein excretion. Creatinine levels are used to check the completeness of collection since creatinine excretion tends to remain stable in a particular patient provided the lean body weight does not change. Normal creatinine excretion rates for adults are 14-26 mg/kg/day in men and 11-20 mg/kg/day in women. As can be seen from our study, quite a significant proportion of 24 hours urine samples are inappropriately collected. However, this figure is much less as compared to that mentioned in many other international publications. Statistics for under collection of urine samples quoted in studies done previously range from 6.45% to 37%. As an example, a study done on 381 patients in USA found out that 24 hours sample collection was abnormal in 50.7% patients. However, this figure included over collected samples as well. Moreover, the authors used higher cut-off limits to define under-collection (<18 mg/kg for males and <15 mg/kg for females in contrast to <14 mg/kg for males and <11 mg/kg for females used here), meaning thereby that this figure would have been lower if they analyzed the way it has been done in this study. Similarly, another study done at Maryland USA found that 30% samples were undercollected. In a Jordanian study, 23.5% samples rejected due to inadequate collection. Another study done on patients with lupus nephritis found inadequate collection in 21.8% samples. A unique feature was that the authors adjusted the cut-off limits for age of the patients in addition to body weight and gender. Using the same operational definitions as this Hong Kong based study, Yadav et al documented 11.5% under-collection rate in a Nepali cohort. This is perhaps one of the few studies that describes results comparable to this one.

Assessment of adequacy of 24 hours urine collection in Pakistani population has not been adequately addressed in local literature, especially so in the recent years. Only a very few publications can be retrieved from the internet. In the presence of alternatives like spot urine albumin: creatinine or protein: creatinine ratios, it is possible that this might be an indication of a decreasing frequency of tests performed on timed collections.

Jafar et al conducted a study in 2001-2002 on patients aged ≥40 years in Karachi in which under-collected 24 hours urine samples were excluded from analysis. Using lower normal limits for creatinine excretion of 9mg/kg in men and 7mg/kg in women, 33 of the 295 samples were rejected, thereby giving a figure of 11.2% for under-collections. The rates are less than ours but then, the cut-off limits are also lower.

A significant risk factor for under-collection of 24 hours urine samples identified in this study was the level of education. For obvious reasons, less educated patients were more likely to submit under-collected samples because of problems with understanding the instructions and following them appropriately. All of our patients were physically independent and had manual dexterity. Even then, rather strangely, patients in the older age group collected samples better than the younger ones. One possible explanation is that patients in the younger age group are socially more active and also need to go out to work for prolonged periods during the day. This might result in inadequate collection as some of the times the void urine may deliberately be discarded.

This study has highlighted the fact that under-collection of 24 hours urine samples is a significant problem encountered in clinical practice. In addition to being a stress and inconvenience for the patient, this adds to the burden on already limited health care facilities available by wasting human effort and physical resources. These alarming figures also call for a unified effort on part of the doctors and paramedics to explain the procedure in detail to the patients and their attendants.

**CONCLUSION**

Under-collection of 24 hours urine samples is not so uncommon, seen more likely in younger and less educated patients. Adequacy of 24 hours urine
samples should always be assessed by measuring creatinine levels before further analyzing the sample. Moreover, considering the high frequency of inadequate collections, other methods for calculating proteinuria should be used preferentially.

REFERENCES


AUTHOR’S CONTRIBUTION

The sole author (ARA) has made substantial contributions to the manuscript in conception and design, acquisition of data, drafting the manuscript and final approval of the version to be published. Author agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

CONFLICT OF INTEREST

Authors declare no conflict of interest

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