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EVALUATION OF SEMI-QUANTITATIVE METHODS FOR PROTEIN AND SUGAR ESTIMATION IN URINE

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ABSTRACT

Urine examination is routinely done for various conditions. This study compares the accuracy of semi quantitative methods of protein and sugar in urine as shown by their agreement with the quantitative estimation. Hundred randomly collected samples of urine were analysed for levels of protein and sugar. Protein estimation was done by dipstick and sulphosalicylic acid method (SSA) and sugar by dipstick and Benedict's semi-quantitative methods. Kappa analysis was done to assess the agreement of these semi quantitative methods with the quantitative estimation. Neither of the two tests for urine protein, dipstick or SSA, showed good agreement with the quantitative estimation (Kappa coefficient 0.26 and 0.07 respectively). However, the dipstick was significantly better than SSA (p‹0.05). for urine sugar, both dipstick and Benedict's tests showed good agreement with the quantitative estimation (Kappa coefficient 0.78 and 0.84 respectively). The difference between them was insignificant. Results demonstrate that for urine protein, dipstick or SSA show poor agreement with quantitative values. For urine sugar estimation, Benedict's semi quantitative test shows good agreement with the quantitative values and it is as good as dipstick methods.

KEYWORDS: Urinanalysis, semi quantitative methods, dipsticks.

INTRODUCTION

Urine examination, for diagnostic purposes, was used even in the arhaic cultures. In fact, by the Middle age, inspection of urine became the only commonly used diagnostic method. The matula, a transparent bulbous urine glass became the symbol of doctors' status and recognition. The gesture of the doctor holding the urine glass against light became the epitome of Middle age physician. This posture was captured in a number of painitngs and drawings of the period. Surprisingly, urine examination has withstood all the sophisticated scientific advances made since then. It remains the most important preliminary investigation even in the most advanced hospitals in the world. The only change that has occurred over the years is in the methodology of the tests. The more time consuming manual methods have been replaced by dipsticks, which are available for virtually every non-soluble analyte of urine and now we have semi-automated and automated urine chemistry analysers. Laboratorians, especially in government set-ups, have to do some tough decision-making. On one hand is the corporate sector making us believe that the latest technologies are much more sensitive, cost effective and less time consuming and on the other is the hospital administration which, more often than not, feels that using newer technologies, especially for simple routine tests, might be a costly affair. We must, however, find ways and means to assess the efficacy and utility of various technologies objectively. It is also important in our struggle to balance the increasing demands and decreasing resources so that we can rethink and re-engineer the laboratory services in a need based manner. The present analysis was carried out to objectively analyse the commonly used semi-quantitative methods for sugar and protein estimation.

MATERIALS AND METHODS

Hundred randomly collected samples of urine were analysed for levels of sugar and protein. In all these samples, protein estimation was done by dipstick strips and sulphosalycylic acid turbidometric method (SSA). Similarly urine sugar was analysed by the same dipsticks and the Benedicts's qualitative / semi quantitative method. The accuracy of these semi quantitative methods was assessed in comparison to the quantitative estimation of these components on a semi- automatic biochemistry analyser. Comparison was also drawn between the cost and time factor. Kappa analysis was done to assess the agreement of these semi quantitative methods with the quantitative estimation.

RESULTS

The observed and chance expected agreements between urine protein dipstick and quantitative estimation were 0.62 and 0.49 respectively. Kappa coefficient was 0.26 with the standard error of 0.07. When the SSA for urine protein was compared with quantitative estimation, observed and chance expected agreements were found to be 0.32 and 0.27 respectively. Kappa coefficient was 0.07 with standard error of 0.04 Neither of the two tests for urine protein, dipstick or SSA, showed good agreement with the quantitative estimation. However, the dipstick was significantly better than SSA (Table 1). In the case of dipstick for urine sugar, the observed and chance expected agreements with quantitative estimation were 0.97 and 0.87 respectively. Kappa coefficient was 0.78 with the standard error of 0.06. When the Benedict's tests for urine sugar were compared with quantitative estimation, observed and chance expected agreements were found to be 0.98 and 0.88 respectively. Kappa coefficient was 0.84 with the standard error of 0.07. Both dipstick and Benedict's tests for urine sugar showed good agreement with quantitiative estimation. The difference between them was insignificant (Table 2). The cost of a single dipstick used in this study was Rs. 6.50 and both sugar and protein estimation were carried out with the same dipstick. The cost of the reagents used for SSA worked out to be about 80 paise and that for Benedict's was approximately 1.60 paise for each test. The time taken for reading dipstick results was 30 seconds (glucose) to 60 seconds (protein). SSA results were after 10 minutes and Benedict's after 2 minutes.

Table 1: Comparison between dipstick and SSA tests for urine protein (using their agreement with quantitative method).

Dipstick's agreement with quantitative		SSA's agreement with quantitative		'Z'	'Y'
Kappa coefficient	SE of Kappa	Kappa	Se of Kappa coefficient		(Two-tailed)
0.26	0.07	0.07	0.04	2.36	0.019 (Significant)

Table 2: Comparison between dipstick and Benedict's tests for urine sugar (using their agreement, with quantitative method).

Dipstick's agreement with quantitative		SSA's agreement with quantitative		'Z'	'Y'
Kappa coefficient	SE of Kappa	Kappa coefficient	SE of Kappa coefficient		(Two-tailed)
0.78	0.06	0.84	0.07	0.65	0.516 (Significant)

DISCUSSION

The presence of increased amounts of protein in the urine can be an important indicator of renal disease Urine proteins mainly comsist of albumin and globulin. Because albumin is more readily filtered than the globulins, it is usually abundant in pathological conditions. Therefore the term albuminuria is often used synonymously with proteinuria. Normally, the glomeruli prevent passage of protein from the blood to the glomerular filtrate. Therefore, the presence of protein in the urine is the single most important indicator of renal disease. Over the years, many tests of renal significance have been adapted for use on strips of cellulose that have been coated or impregnated with reagents for the concerned analyte. The dipstick test for total protein comprises of a cellulose test pad impregnated with tetraboromophenol blue and a citrate pH 3 buffer. The reagent is most sensitive to albumin and less sensitive to globulins, Bence Jonce proteins, mucoproteins and haemoglobin. SSA method on the other hand will also detect globulins and Bence Jonce proteins. In our study, a comparison of reagent strips and the SSA method showed that though neither dipstick nor SSA showed good agreement with the quantitative protein estimation, dipstick was significantly better than SSA method. Probably that is because most of the proteinurias detected are albuminurias and reagent strips are known to be more accurate when only albumin is present. Acid precipitation tests might be of relevance when proteinurias other than albuminurias are suspected. To put it simply, dipsticks have greater diagnostic utility in glomerular proteinurias while SSA's relevance is more in tubular proteinurias.

As far as screening for glucosuria is concerned, the Benedict's copper reduction test is not specific for sugars and is affected by most reducing substances if they occur in large quantitities. It is therefore also useful in detection of sugars other than glucose. Dipstick reagent pad for glucose estimation is impregnated with glucose oxidase, peroxidase, potassium iodide and blue dye. The reaction employs glucose oxidase and peroxidase produce hydrogen peroxide, which is subsequently reduced with concurrent oxidation of potassium iodide to release iodine. Free iodine blends with the background colour to produce a range of colours from green to dark brown. As a screening test, this glucose oxidase test will not detect increased levels of galactose or other sugars in urine. It is therefore important that a copper reduction test be used for young pediatric patients. In those instances when the copper test is positive and the glucose oxidase is negative, glucosuria is ruled out and investigations for other sugars should be carried out. These cases, however, are very few. In a routine setting, our study show that both dipstick and Benedict's tests show good agreement with the quantitative estimation and the difference between them was also insignificant.

If the cost of the test are compared, manual method obviously appear significantly cheaper but the hidden cost of glassware, burners and manpower should be borne in mind. Time consumption is distinctly more for manual methods of semi quantitative estimation.

CONCLUSION

Though dipsticks will suffice in most clinical settings, SSA and Benedict's test will be relevance in some special circumstances and cannot be set aside from the laboratory bench top. Dipsticks are more sensitive to albumin and less to globulins, mucoproteins, haemoglobin and Bence Jonce proteins. SSA will therefore have more diagnostic utility if later are to be detected. Benedict's test is affected by all reducing sugars while dipsticks only by glucose. Benedict's will be of relevance in young paediatric patients for detecting other sugars. As resources become constrained it is increasingly important to monitor the reliability and cost-effectiveness of different methods so that a judicious use is exercised as per the needs of clinical situation, clinical setting, financial and manpower resources available.

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