A Workshop on Urinalysis and a Survey on Urine Microscopy Among Kidney Centers of Iran

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Urinalysis is a mandatory diagnostic tool for the evaluation of patients with kidney diseases. A workshop on urinalysis was held for nephrologists in Isfahan, Iran, on October 11-12, 2012. After the presentation of the results of a survey of the nephrology centers of Iran on urine microscopy, the most important aspects of urinalysis were presented and discussed. These included the following: (1) urinalysis by dipstick, which provides results in a few seconds, is simple to use, has a low cost, and is used worldwide for screening purposes, in spite of some limitations; (2) measurement of proteinuria by 24-hour urine collection, which still represents the reference method in spite of limitations due to frequent over or under collection errors; (3) protein-creatinine ratio in a random urine sample, which is recommended by international guidelines as an alternative to the measurement of 24-hour protein excretion; (4) microalbuminuria, which is seen as a marker of systemic endothelial damage; and (5) the urinary sediment, which is underused even among nephrologists in spite of the relevant diagnostic information it can supply in a wide spectrum of kidney diseases.

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SPECIAL REPORT

INTRODUCTION

Urinalysis is a mandatory diagnostic tool in the evaluation of patients with kidney diseases. In nephrology practice, besides basic information (ie, pH, specific gravity, and semiquantitative measurement of albumin, hemoglobin, glucose, leukocyte esterase, nitrites, bilirubin, and urobilinogen, which is usually obtained by dipstick),[1] more detailed information is required. This includes the quantitative measurement of protein excretion, expressed either per 24 hours or as protein-creatinine ratio on a random sample, and in some settings, microalbuminuria.

Urinalysis also includes urinary sediment examination, which can supply valuable information in a wide spectrum of clinical conditions.[1] In spite of this, urinary microscopy is too often performed without the proper methodology, instrumentation, and capability of identification of the particles, in general laboratories and even among nephrologists.[2-5] Moreover, it is frequently underused, although recent publications call for a reappraisal among nephrologists of this quick and inexpensive test.[6]

In this article, we describe a workshop on urinalysis for nephrologists, which was held in Isfahan, Iran, on October 11-12, 2012, in which, after the presentation of the results of a survey of Iranian renal centers on urine microscopy, the basic and more sophisticated aspects of urinalysis were presented and discussed. The workshop was sponsored by Roche-Iran (Tehran, Iran). The participation was by invitation and free of charge. The scientific program was drawn up mainly by the local guest (author, SS). The workshop was preceded by an e-mail distribution to the nephrology

centers in Iran of a questionnaire on some aspects of urine microscopy.

RESULTS AND DISCUSSION

Venue and Participants

The workshop was held at Abulfaz Charity Centre of Isfahan. It included several sessions (Table 1), all based on presentations and discussions. Altogether, there were 25 participants, all nephrologists from different nephrology centers (7 participants from 7 hospitals of Tehran, 13 from Isfahan, and 1 each from Kashan, Kermansha, Mashad, Tabriz, and Yazd).

Presentation of Survey on Urine Microscopy

The questionnaire was sent to all 20 nephrology centers in Iran, 18 of which responded, either to all questions or only to some of them. From the answers received (Table 2), the number of urine sediments examined monthly in the nephrology centers varied from 20 and 1000 per month, and the urine sediment examination was being requested for only 50% of hospitalized patients and 27.8% of outpatients. For 44.4% of centers, the urine sediment examination was being done in central laboratories, while in the remaining centers, nephrologists were also involved, especially senior nephrologists, who were also the main providers of the training on the subject. The majority of centers (61%) would give some instructions to patients on how to collect urine, the first urine of the morning, collected after cleaning of external genitalia, being the sample most frequently used. The questions on the handling of the urine samples showed that there were large differences between centers in centrifugation (for both volume of urine centrifuged and duration of the procedure) and that removal of the supernatant urine after centrifugation, resuspension of the centrifuged sediment, and transfer of the urine to the glass slide would be carried out mostly through nonstandardized procedures (the so-called pouring-off). Finally, the questionnaire showed that only bright-field microscopy was being used, filters for polarized light were rarely available, and one-third of the centers did not have the proper microscopic magnifications.

Presentation on Urinalysis by Dipsticks

These are plastic sticks bearing different pads impregnated with different reagents to test several urinary parameters at the same time (ie, pH, specific gravity, albumin, haemoglobin, glucose, leukocyte esterase, nitrites, bilirubin, urobilinogen, and ketons). After the dipstick is plunged into the urine for few seconds, the color of each pad is matched with the color reference spectrum shown on the stick box, the results being expressed according to a semi-quantitative scale.

Dipsticks provide results in few seconds and are simple to use, have low cost, and are used worldwide for screening purposes and as first-line test. Moreover, the matching of dipstick findings with urine microscopy findings increases the accuracy of urinalysis.[7] On the other hand, dipstick testing is associated with semi-quantitative results only, which makes it susceptible to various interference factors, and urine discoloration, as well as time sensitivity for reading, which may be variable according to the dipstick brand.[1,8]

Of note, the pad for proteins is almost exclusively sensitive for albumin, for which reason both

Table 1. Scientific Program of the Workshop

Topic	Presenter (City, Country)
Results of a questionnaire on urinary microscopy in Iranian nephrology centers	Maryam Hami (Mashad, Iran)
Urinalysis by dipstick	Nader Majelan (Yazd, Iran)
Urinary protein measurement by 24 hours	Abdolamir Atapour (Isfahan, Iran)
Urinary protein measurement by urinary protein/creatinine ratio	Sharzhad Shahidi (Isfahan, Iran)
Microalbuminuria	Maryam Hami (Mashhad, Iran)
The urinary sediment The particles and their clinical meaning The urinary sediment in: - glomerular diseases - acute interstitial nephritis - acute kidney injury - kidney transplant (with focus on BK virus reactivation) - clinical cases (isolated microscopic hematuria; Fabry disease; cytomegalovirus infection; BK virus nephropathy)	Giovanni B Fogazzi (Milano, Italy)

Table 2. Main Questions and Results of the Survey

Question	Response, n	Answer, n
In your nephrology center, how many urinary sediments are examined on average per month?	18	173 ± 262 (20 to 1000)
Is urine sediment examined for all your renal patients?	18	
Inpatient		
Yes		9 (50.0)
No		6 (33.3)
Some		3 (16.7)
Outpatient		
Yes		5 (27.8)
No		5 (27.8)
Some		8 (44.4)
Where is urinary sediment examination performed?	18	
In central laboratory only		8 (44.4)
Both in central laboratory and in nephrology center		10 (55.6)
Who does the examination of urinary sediment in nephrology centers?	10	
Senior nephrologists		4 (40.0)
Junior nephrologists		1 (10.0)
Fellows		1 (10.0)
All the above on rotation		4 (40.0)
How were the above professionals trained on urine microscopy?	9	
By senior nephrologists		5 (55.6)
By courses		3 (33.3)
By themselves		1 (11.1)
Do you give instructions to your patients on how to collect urine?	18	
No		7 (38.9)
Yes		11 (61.1)
Which procedures do you suggest for urine collection?	11	
First urine of the morning		8 (72.7)
Second urine of the morning		3 (27.3)
Cleaning of external genitalia with water		11 (100)
Cleaning of external genitalia with disinfectants		0
How do you handle urine?	11	
Volume of urine centrifuged, mL		13.0 ± 12.3 (10 to 50)
Time of centrifugation, min		8.0 ± 3.5 (5 to 15)
Removal of supernatant urine after centrifugation by:	10	
Pouring off		8 (80.0)
Pasteur pipette or pump		2 (20.0)
Resuspension of sediment by:	11	
Shaking or finger flipping of the tube		10 (90.9)
Pasteur pipette or pump (fixed volume)		1 (9.1)
Transfer of resuspended sediment to the slide by:	11	
Pouring off		4 (36.4)
Pasteur pipette or pump (fixed volume)		7 (63.6)
Which microscope do you use for the examination of urinary sediments?		
Bright field microscope	11	8 (72.7)
Polarized light when needed	3	3 (26.3)
Use of low + high magnification (100x or 160x + 400x)	9	6 (66.6)

*Values in parentheses are percentages for proportions and range for mean values.

immunoglobulin light chains and light chains fragments (typical of overflow proteinuria caused by monoclonal gammopathies) and tubular proteins (typical of tubulo-interstitial renal diseases) are missed. Moreover, detection threshold of dipstick for albumin is at 200 mg/dL to 300 mg/dL, which misses the detection of microalbuminuria.

Presentation on 24-hour Protein Excretion

Precipitation methods, dye binding techniques,

and biuret methods can all be used to quantitate urine proteins. Of these, biuret methods are today considered as the most reliable, since they have the same sensitivity for all proteins and the minimal interference from drugs, radiographic contrast media, and colored metabolites.

The 24-hour urine collection is the reference (gold standard) method to quantitate proteinuria. This is because it averages the variation of proteinuria due to circadian rhythm (peak level at midday and in the afternoon, nadir level overnight and in the morning) and is the most accurate method for the monitoring proteinuria during treatment. However, it is impractical in several settings (eg, children, elderly, uneducated patients, and outpatients), it is frequently exposed to overcollection or undercollection errors, and is greatly influenced by variations in water intake and diuresis volume.[9,10] For all these reasons, urine collection is today seen as a chore for the patient and a nuisance for the doctor, who has to give simple but definite instructions (possibly written) to the patients and check whether the collection has been done in a correct way.[11]

Presentation on Protein-Creatinine Ratio

This method, which is recommended by international guidelines as an alternative to 24-hour urine excretion measurement,[12] offers the following advantages: the urine sample is easy to obtain, the protein-creatinine ratio is not influenced by variations in water intake and rate of diuresis, and many studies on a wide range of patients groups have demonstrated a close correlation with 24-hour protein excretion. However, this method too has some limitations. In fact, protein-creatinine ratio on a random urine sample may be influenced by the timing of the sample, due to daily circadian rhythm of proteinuria compared to a relative constancy of 24-hour creatinine urine excretion; it can be associated with over estimation of proteinuria in females and in the elderly, as a consequence of reduced urinary creatinine excretion due to reduced muscle mass; and it may show a poor correlation with 24-hour excretion at moderate to high levels of proteinuria.[13,14]

A meta-analysis performed on 16 studies including different types of renal patients concluded that protein-creatinine ratio on a random urine sample could be used to rule out patients with proteinuria, a fact which would reduce the number of unnecessary 24-hour urine collections, while the finding of proteinuria above the cutoff level would require a full 24-hour quantitation.[15]

Presentation on Microalbuminuria

Microalbuminuria is defined as an increased albumin excretion from 30 mg/d to 299 mg/d, that persists over a 3- to 6-month period.[16,17] In diabetics and maybe in the general population, microalbuminuria identifies the subjects who are at increased risk of chronic kidney disease, cardiovascular morbidity and overall mortality. In addition, in diabetic patients it identifies those who are at increased risk of developing overt diabetic nephropathy.[16,17] This is because microalbuminuria reflects a state of generalized impairment of vascular function, with the kidney being a window through the vasculature in other tissue compartments.[17]

Semi-quantitative dipstick tests are available to screen for albuminuria such as Microalbutest, Albuscreen, Clinitek Microalbumin Dipstick and Micral-Test II test strip.[18] However, once microalbuminuria is found by dipstick, a standard quantitative method must be used for confirmation. These methods consist of immunoturbidimetry, nephelometry, radioimmunoassay enzyme-linked immunosorbent assay, or high performance liquid chromatography.[19] Due to its great simplicity, immunoturbidometry is the method most frequently used.

Although the 24-hour urine collection was initially considered the gold standard method for the detection of microalbuminuria, today the use of morning urine samples is preferred, since it prevents urine volume variations which can occur during the day.[9]

Presentations on Urinary Sediment

The clinical utility of urinary sediment examination was extensively dealt with during the workshop (Table 1). The presentation on particles described the morphology, as seen by phase contrast microscopy, and clinical meaning of the elements, which can be found in the urinary sediment. Special attention was given to dysmorphic and isomorpich erythrocytes, whose identification allows to distinguish glomerular from nonglomerular hematuria; renal tubular epithelial cells, which are a marker of acute renal

tubular damage; cellular casts, which indicate a renal origin of the cells they contain; and crystals due to drugs, which are poorly known and can be associated with acute kidney injury.[8]

The presentation on glomerular diseases reported on the background knowledge about the subject and the results of a prospective study, which is still in progress at Ospedale Maggiore Policlinico of Milano. The first results of this study, published in 2005 showed how the urinary sediment examination, performed in an accurate and standardised way, could distinguish proliferative from nonproliferative glomerular diseases, and how some significant correlations could be found between the presence of some urinary particles and some lesions at renal biopsy.[20]

The presentation on acute interstitial nephritis also described the available information on the subject and the results of a recent retrospective study done in Milano.[21] This showed that erythrocytic casts, which are considered extremely rare in acute interstitial nephritis, were found in the urine of 6 of 21 patients (28.5%) with acute interstitial nephritis from different causes. This led to the conclusion that the finding of erythrocytic casts in the urinary sediment can no longer be used as a criterion to rule out the diagnosis of acute interstitial nephritis.

The presentation on acute kidney injury reported on the most recent results of the literature, which demonstrate that pre-renal acute kidney injury can be distinguished from acute kidney injury from acute tubular necrosis also on the basis of urinary sediment findings. In a prospective study including 231 hospitalized patients with acute kidney injury, those with acute tubular necrosis had a significantly higher urinary sediment score based on the presence and number of granular casts and renal tubular epithelial cells.[22] The same score was also found to be a more reliable predictor of acute kidney injury worsening than changes of serum creatinine from baseline.[23]

The presentation on kidney transplant described the value of urinary sediment examination in diagnosing the reactivation of polyomavirus BK, which can lead to a specific nephropathy with renal dysfunction up to the loss of the graft. In this condition, urinary sediment shows the presence of the so-called "decoy cells," which displays 4 main types of nuclear changes namely, ground glass or gelatinous appearance; intranuclear inclusion surrounded by a clear halo, as in cytomegalovirus infection; vesicular nucleus; and clumped chromatin.[24] While it is stated that these changes are best seen on alcohol-fixed samples stained with Papanicolaou,[24] some authors have demonstrated that they can also be identified by phase contrast microscopy on wet, centrifuged samples.[25,26] Of importance, this last approach is technically simple, quick, inexpensive and easily achievable by nephrologists themselves. Clinical cases demonstrated the clinical utility of urinary sediment examination in a wide spectrum of conditions (Table 1).

CONCLUSIONS

The workshop dealt with several aspects of urinalysis, all of which offer more than one hint for discussion. Dispticks, in spite of all the limitations described above, still represent an important first-line tool to rule in or rule out the presence of a renal disease. However, in order to obtain the best results the performances, limitations, interfering factors and features of reading of each pad of dipstick must be known by the operator.[1,8]

The measurement of proteinuria on 24-hour urine collection is a method widely criticized today, and there is no doubt that it may be impractical and inaccurate for several reasons. However, it cannot be forgotten that when the urine collection is performed correctly it still represents the reference method. In order to avoid undercollection or overcollection, written and simple instructions on how to collect urine should be given to patients,[11] in which also the aim and the importance of a correct collection are explained and stressed. Another practical possibility could be to reserve 24-hour urine collection only for those patients who appear to be able and keen to do that.

The measurement of protein-creatinine ratio on random urine sample offers undoubtedly major advantages over the 24-hour urine collection. For all these reasons it is recommended by international guidelines[12] and is today used worldwide. However, also for this method the limitations should be taken into account, especially for patients with reduced muscle mass and high levels of proteinuria.[13-15]

In spite of the massive literature on the subject, the issue on which method to prefer for the measurement of proteinuria is still so open that 2012 International Society of Nephrology guidelines on glomerulonephritis states that "there is currently

insufficient evidence to preferentially recommend 24-hour, shorter-timed, or spot urine collections for proteinuria management in glomerulonephritis.[27]"

Microalbuminuria reflects a state of generalized impairment of vascular function, which depends on several factors such as hyperglycemia, poor arterial hypertension control, dyslipidemia, high-risk lifestyle, etc.[17] From this it appears that microalbuminuria does not indicate a renal disease but rather a global imbalance, which needs an integrated therapeutic approach.

Urine sediment examination, as demonstrated during the workshop, is of diagnostic utility in several renal conditions, from isolated microscopic hematuria to glomerulonephritis, acute interstitial nephritis, acute kidney injury and renal transplantation. All this should be considered without forgetting that in an appropriate clinical setting, even negative urinary findings have a diagnostic value.

We consider of great importance the results of the questionnaire which was sent to the Iranian nephrologists in view of the workshop. Altogether, it appears that in Iran urine microscopy is underused by nephrologists, the handling of samples is carried out mostly through non standardized methods and the samples are examined without the proper microscopic equipment, with easily imaginable influence on the quality of results.

All this leads to the question whether this situation can be improved or not. In this respect, it must be considered that a urine microscopy program requires relatively small financial and human investments, especially when compared to other techniques. In fact, urine microscopy requires very ordinary equipment and no consumable reagents, the only major financial burden being for the microscope.[7] This must be of high quality, equipped with phase contrast and polarized light filters, 2 magnifications (eg, 100 × or 200 × and 400 ×), and possibly, also with a digital camera, even a low-cost one,[28] for documentation, circulation of images, and educational purposes. The fact that such a program is today possible in Iran is well demonstrated by the experience of Mashhad University of Medical Sciences, where an advanced urine microscopy program, based on standardized procedures for the handling of urine samples and the purchase of a proper microscope, has recently been introduced by the authors (MH).

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CONFLICT OF INTEREST

None declared.

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