UriSed as an Alternative to Phase-Contrast Microscopy in the Differentiation between Glomerular and Non-Glomerular Hematuria

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SHORT COMMUNICATION

UriSed as an Alternative to Phase-Contrast Microscopy in the Differentiation between Glomerular and Non-Glomerular Hematuria

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SUMMARY

Background: **Differentiation between glomerular and non-glomerular hematuria by observation of the erythrocyte morphology using phase-contrast is a time-consuming and labor-intensive procedure that requires skilled personnel. This paper has the purpose to evaluate the performance of UriSed (also called sediMAX® in some countries) as an alternative to the phase-contrast microscopic analysis of erythrocyte morphology.**
Methods: **312 urine samples with hematuria were analyzed by UriSed and by phase-contrast microscopy. Based on the presence of codocytes and/or acanthocytes, samples were classified as non-glomerular and glomerular. Kappa correlation was used to assess the agreement between both methods.**
Results: **Our data showed excellent agreement between erythrocyte morphology analyzed by both methods (r = 0.974, kappa = 0.9484, p < 0.001) with only 8 samples presenting discordant results.**
Conclusions: **UriSed proved to be a precise and accurate alternative to the gold standard phase-contrast microscopy.**
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KEY WORDS

hematuria, phase-contrast microscopy, automated sediment analysis

INTRODUCTION

Hematuria is defined as the abnormal presence of red blood cells (RBC) in urine and can be classified as gross (macroscopic) or microscopic hematuria. Macroscopic hematuria is easily perceived by individuals. This situation is often alarming and motivates the patient to look for medical attention. On the other hand, microscopic hematuria is usually asymptomatic and is often an incidental finding during a routine urinalysis. It is one of the most common urinary abnormalities and may be observed in up to 21% of the population [1]. Hematuria may be transient or persistent and may be due to multiple causes including glomerular and non-glomerular disorders [2]. In the 80s, studies conducted by Birch and Fairley demonstrated the possibility of differentiating glomerular and non-glomerular hematuria by the observation of erythrocyte morphology by phase-

Table 1. Comparison between UriSed and phase-contrast microscopy.

		Phase-contrast microscopy	
		dysmorphic RBC *	isomorphic RBC
UriSed	dysmorphic RBC *	164	3
	isomorphic RBC	5	140

*** - presence of codocytes and/or acanthocytes.**
Agreement = 0.9744, kappa = 0.9484, p < 0.0001.

contrast microscopy [3].
Among several erythrocyte morphologies observed in the urinary sediment, two of them are highly significant for the diagnosis of glomerular hematuria: acanthocytes and codocytes. Acanthocytes are doughnut-shaped cells with one or more blebs whereas codocytes are target-like shaped or doughnut-shaped cells without membrane protrusions or blebs. The latter are the predominant cells in patients with glomerular disease although acanthocytes are considered more specific for the differentiation between glomerular and non-glomerular hematuria [4-7]. Phase-contrast microscopy is considered the golden standard for detecting dysmorphic erythrocytes. However, it is a time-consuming and labor-intensive procedure that requires skilled personnel.
UriSed (also called sediMAX® in some countries) is a walk-away automated urine sediment analyzer based on microscopic examination of urine samples (KOVA method). This image based analyzer automatically performs all steps of the traditional manual microscopy providing whole-field and high-definition images that are displayed on-screen and can be reviewed by an experienced analyst and manually edited, if necessary [8].
This paper has the purpose to evaluate the performance of UriSed as an alternative to the phase-contrast microscopic analysis of erythrocytes morphology.

MATERIALS AND METHODS

We evaluated 312 clean-catch midstream urine samples from patients (43% men and 57% women) with hematuria caused by several clinical conditions. Their age ranged from 01 to 93 years old (median = 44 years). Hematuria was defined as an erythrocyte counting of more than 5 cells/high power field (hpf).
All mid-stream urine samples were examined within 2 hours after voiding by phase-contrast microscopy and by a fully image-based automated sediment analyzer (UriSed - 77 Elektronika Kft, Budapest, Hungary).
Initially, samples were analyzed by UriSed and all the images reviewed by an experienced analyst. Parallelly, the urine samples were centrifuged (10 mL, 5 minutes, RCF = 400) and the sediment (0.5 mL) was placed on a slide and examined under a coverslip by phase-contrast microscopy. Erythrocyte morphology was analyzed by both methods by different observers. Differentiation between glomerular and non-glomerular hematuria was based on the presence of particular erythrocyte morphologies. Presence of codocytes and/or acanthocytes defines glomerular hematuria whereas their absence classifies this form of hematuria as non-glomerular. Kappa correlation was used to assess the agreement between both methods.

RESULTS

Our data showed excellent agreement between erythrocyte morphology analyzed by both methods (r = 0.974, kappa = 0.9484, p < 0.001, Table 1).
Figures 1 and 2 show erythrocyte morphology observed by phase-contrast microscopy and by UriSed.
From 312 samples, 140 of them (45%) presented isomorphic erythrocytes and hematuria was classified as non-glomerular by both methods whereas in 164 samples (52.5%) we observed the presence of codocytes and/or acanthocytes by phase contrast microscopy and by UriSed being classified as glomerular hematuria. Only 8 samples (2.5%) had discordant results. Five of them revealed the presence of codocytes by phase contrast microscopy which was not displayed on UriSed. On the other hand, 3 samples classified as non-glomerular by phase contrast microscopy presented codocytes on UriSed images. It is noteworthy that there was no disagreement on the observation of acanthocytes.

DISCUSSION

There is no doubt about the importance of urinary sediment and research on erythrocyte dysmorphism in the evaluation of patients with hematuria. Similarly, it is believed that phase contrast microscopy is superior to bright-field microscopy for detecting dysmorphic erythrocytes [5,9]. Nevertheless, some authors prefer the bright-field microscopy as it is accessible in every fairly well equipped laboratory and it would be easier to perform and less time-consuming compared with phase-contrast microscopy [10-12].
Significant inter-observer variation in the identification and interpretation of urine structure is an important lim-

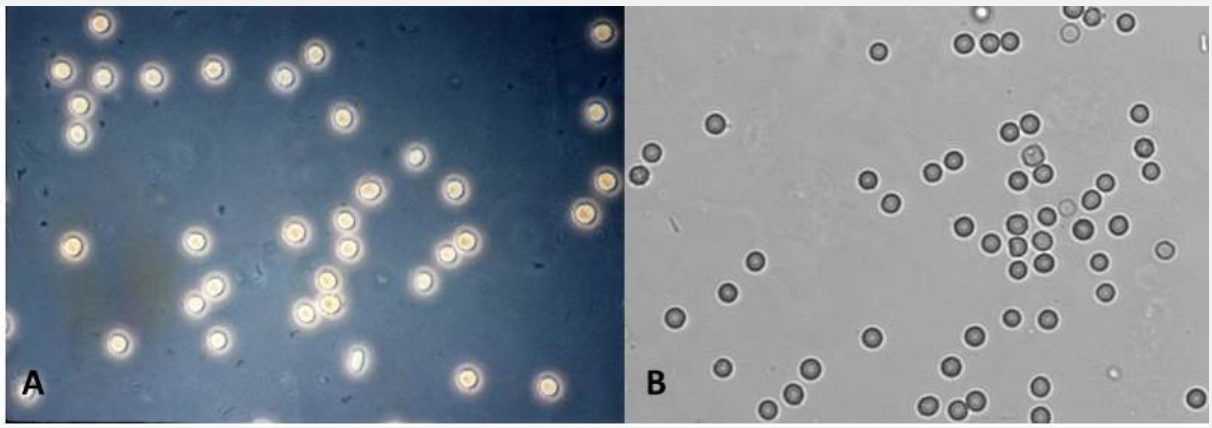

Figure 1. Non glomerular hematuria: isomorphic erythrocytes.

A - phase contrast microscopy, B - UriSed.

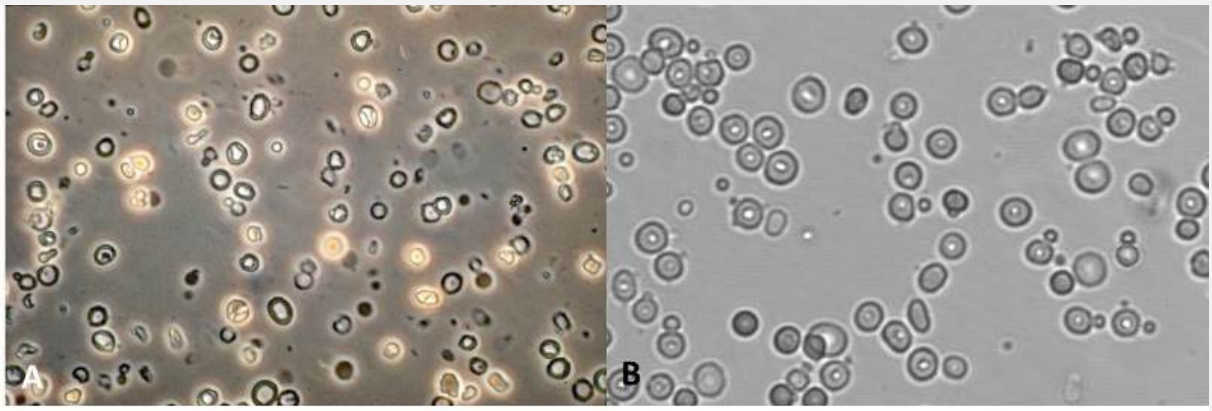

Figure 2. Glomerular hematuria: codocytes and acanthocytes.

A - phase contrast microscopy, B - UriSed.

itation for both forms of microscopy [13,14]. As stated by Huussen et al. [11], after sufficient training of the analysts, it is possible to achieve excellent inter-observer correlation although this expertise is hard to obtain. Apropos, it is important to highlight that RBC may present several morphological changes which do not characterize glomerular hematuria. The presence of urinary sickle cells, anisocytes, poikilocytes, dacryocytes, and elliptocytes correspond to several other clinical conditions [15].

For a reliable differentiation between glomerular and non-glomerular haematuria, significant experience is necessary in evaluating urinary sediment. Some studies show that RBC morphology analysis can be performed on samples previously fixed and analyzed under phase-contrast [16] or bright field microscopy [11]. Another study describes the use of Papanicolaou slides to differentiate glomerular and non-glomerular hematuria [12].

The use of fixation techniques allows the samples to be re-analyzed and even to be sent for consultation to a reference laboratory.

Another difficulty consists in the fact that the criteria for urinary dysmorphic erythrocytes are not universally standardized. The first studies attempting to overcome this limitation were based on the evaluation of the RBC volume distribution curves in glomerular and non-glomerular hematuria [17,18].

In the early 2000s image based automated sediment instruments began to be used in the analysis of urine sediment but only recently its use was extended to the analysis of RBC morphology [19]. Fogazzi and Garigali clearly demonstrated that the two types of hematuria can be identified with UriSed [20]. The same was observed in this study and, as these authors, we also evaluated RBC morphology by reviewing the images displayed on-screen. Regarding our few discordant results between phase-contrast microscopy and UriSed, it was not possible to accurately establish the cause of hematuria in order to clarify which methodology was more accurate.

It should be highlighted that the use of a system that photographs and stores high-definition images makes it possible to have a second opinion by re-examining the samples if necessary and also provides a powerful training tool for students and laboratory staff.

In summary, UriSed proved to be a precise and accurate alternative to the gold standard phase-contrast microscopy. We are convinced that the adoption of UriSed as an alternative to phase-contrast microscopy improves workflow and may reduce turnaround time.

Declaration of Interest:

The authors declare that there are no conflicts of interest.

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