COMPARATIVE ANALYSIS OF STANDARDISED AND NON-STANDARDISED MICROSCOPY METHODS OF URINE SEDIMENTS

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Abstract

The aim of the study – to compare standardised and non-standardised methods of urine sediment microscopy.

The study included 40 urine samples with increased erythrocyte and/or leukocyte and/or protein levels as compared with recommended normal values detected by chemical analysis method and additional 20 urine samples which chemical analysis did not reveal any pathological changes. All 60 samples were tested applying a standardised and non-standardised sediment microscopy analysis.

Keywords: standardized, non-standardised, urine sediment.

Introduction

Urinalysis is usually a part of prophylactic and diagnostic health examination. It consists of assessment of urine physical properties, chemical analysis, and microscopy of urine sediment. Although it is a common examination, it is one of the least standardised examinations in clinical practice. The dipstick method is a preferred and well-known alternative for the rapid diagnosis of urinary tract infections in clinical practice, however it's accuracy is low. The large extent study has demonstrated that diagnosis by dipstick method only has led to unnecessary treatment prescription to the patients to whom the treatment was not medically indicated [1]. Thus, the authors have concluded that manual microscopy is necessary to avoid errors in identifying pathological cells, bacteria, spores, crystals or casts. These methods were found to be equivalent for urine sediment examination except of cast detection. [2]. Moreover, not all laboratories follow the CLSI (Institute for Clinical and Laboratory Standards) recommendations to carry out the urinary sediment microscopic analysis of the samples found to be positive for erythrocytes and/or leukocytes and/or protein by chemical analysis. A large proportion of laboratories still perform non-standardized microscopic analysis, which has been shown by scientists to be inappropriate due to its high uncertainty. In laboratory practice, when the renal epithelium is suspected in the sample, examination of stained urinary sediment should be carried out, however, for economic reasons this is often not done and may be the cause of delayed diagnosis of diseases of the urinary system. This issue is largely discussed in scientific society and efforts have been taken to find the most appropriate method for timely and accurate diagnosis. The aim of our work was to demonstrate the similarities and differences between the standardised and non - standardised urine sediment microscopy methods. The KOVA® urine sediment staining was used as a standardised examination method. The KOVA® method was accredited by CLSI as a standardised method for urinary sediment testing [4]. This method involves urine sediment staining and microscopic examination in a chamber. A hypothesis has been raised that standardized microscopic analysis is more accurate method for detection of urine sediment elements than a non-standardised method. To support this hypothesis, 60 urine samples were randomly analysed by the non-standardized and standardized (KOVA®) methods. Twenty samples showing normal results and 40 samples showing pathological changes after chemical analysis were selected for testing. All data obtained were processed by the IBM SPSS statistical analysis program.

Materials and methods

The traditional non-standardised method is still used in many laboratories, it involves centrifugation of urine sample examination of obtained native urine sediment by light field microscopy of the slide made using an undefined volume of sediment droplet under or even without a coverslip. Such type of testing is not recommended due to its uncertainty and low sensitivity for particle detection. Standardised laboratory methods are necessary to ensure more

accurate analyte detection limits of and to obtain the most accurate result possible [3]. The standardised test is recommended as a routine visual method for detection of elements associated with kidney disease, involving the examination of a slide made using the known volume of centrifuged urine sediment covered with a coverslip.

Tested urine samples were selected randomly. A total of 60 samples were tested, independently of patient's age, gender, social status, the department in which they were treated. Forty selected samples were positive for erythrocytes and/or leukocytes and/or protein after the chemical analysis. And 20 urine samples had no abnormalities. 28 males and 32 females were enrolled in the study. Patients' age ranged from 23 to 97 years. Most of the samples were collected from the patients treated at therapy and orthopaedics departments.

The urinalysis consists of the assessment of physical properties and chemical analysis; and if deviations from the normal values are found, the subsequent decision is taken regarding the necessity of further microscopic analysis of urine sediment. According to the recommendations of the Institute for Clinical and Laboratory Standards (CLSI), a microscopic analysis should be performed of the samples found positive by chemical analysis for at least one or more of the following analytes erythrocytes, leukocytes, protein. Scientific data show that chemical analysis is a much more reliable, comprehensive, and faster diagnostic method that does not require specific skills. And the sensitivity of microscopic analysis is only 38.8 %, this method also requires high time input and microscopy skills [4]. The main drawback of this diagnostic method is the long time period between the collection of the sample and execution of testing. It is important to carry out chemical urine testing with two hours from sample collection, as the test may be inappropriate because of bacterial growth and cell lysis. When examination for WBCs (leukocytes), RBCs (erythrocytes), and other analytes is carried out 120 and 240 minutes after sample collection significant decrease in cell amount is reported [5]. Thus, urine sediment examination should be done within one hour from a sample collection.

After urine collection, physical properties are assessed - quantity, colour and transparency are described. All samples delivered to the laboratory are identified and urine dipstick test is performed. A general urinalysis starts with a chemical analysis carried out in an analyser using the dry chemistry diagnostic urine strips. Before the chemical analysis, it is important to mix the urine well, adding about 10 ml of urine to a centrifuge tube. The tubes and accompanying documents are numbered or barcoded. The specimen then is transferred into the test cartridges and placed in the analyser. When the dry chemistry test results are received from the analyser, the test shall be verified and test results shall be printed. Then the urine samples showing pathological abnormalities (presence of erythrocytes, leukocytes, or protein) and those without any pathological changes were selected. The samples underwent centrifugation at 1500 rpm for 5 minutes to prepare aliquots for non-standardized analysis. The supernatant was aspirated or drained and a residual sediment was left for the examination. An unspecified amount of urine sediment is transferred on a slide and covered with a coverslip.

Urine sediment was examined under a light microscope, the whole preparation was examined under small magnification, that is, using10x (for cast evaluation), and then 40x (for the evaluation all other elements) magnifying lenses. Microscopy was carried out with a lowered condenser.

Subsequently to a non-standardised microscopic analysis of urine sediment, a standardized microscopic examination of the same urine sample was performed according to KOVA INTERNATIONAL recommendations. For this purpose, 12 ml of urine was transferred to KOVA Tubes and centrifuged at 1500 rpm 5 minutes. After centrifugation, 1 ml of urine was taken using the KOVA -petter pipette, and the supernatant above the pipette was discarded. The obtained precipitate was resuspended and stained using KOVA Stain (10 minutes). A drop of 6.6 µl of the precipitate of the stained sample is transferred to a graduated 3mm x 3mm measuring chamber with a glass. This chamber is 0.1 mm deep and holds 0.9 µl of liquid. It's camera consists of 81 small squares sized 0.33 mm x 0.33 mm. One square corresponds to one large magnification field. Microscopic analysis was also performed under a light microscope, the casts were evaluated at low magnification (10x) and all other elements at high magnification (40x). If the preparation was rich in tested elements, the elements were counted in five cell squares. However, if the count of elements was low, the elements were counted in ten cell squares.

Results and discussion

Forty patients with urinalysis results positive for protein and /or erythrocytes and /or leukocytes were included in the study. The samples collected from twenty patients whose chemical urinalysis results did not show the above-mentioned abnormalities were also analysed in the study. All urine samples had to meet one criterion - they should not contain vitamin C. The data were processed by the IBM SPSS statistical program. The criterion for dependent

samples and Pearson correlation with a significance level p = 0.05 were used. Zero and alternative hypotheses were raised prior to this study. The null hypothesis stated that the number of elements detected in the same urine sediment sample applying the non-standardised and standardised test method would be the equal in all measurements. The alternative hypothesis said that the standardised method would detect more elements and with higher precision.

Patients age varied from 23 to 97 years. The mean age was 61.80 ± 19.943 years. 32 (53.3 %) of patients were females and 28 (46.7 %) were males. The majority of samples were obtained from the patients treated at the therapy (26.7 %) and orthopaedics (16.7 %) departments. Non-standardised and standardised microscopic urine sediment analysis was performed on all urine samples. All samples were free of epithelial, leukocyte, erythrocyte, haemoglobin/myoglobin, waxy, fatty casts, cysteine, leucine, tyrosine, cholesterol, hemosiderin or hippuric acid crystals, T. Vaginalis, lipid droplets and atypical cells. The following elements were detected in the urine sediment: hyaline and granular casts, squamous transitional and renal epithelium cells, intact erythrocytes, leukocytes, bacteria, urates, uric acid crystals, triphosphates, amorphous phosphates, spores, sperm and mucus.

Out of 20 urine specimens that were found normal by chemical analysis microscopic analysis revealed abnormalities in 17 (85 %) samples and 3 (15 %) samples were confirmed as having no changes. And in all tested samples (40) with reported pathological findings by the chemical analysis, these findings changes were confirmed by microscopic analysis.

Table 1

Comparison of hyaline and granular cast microscopy results by non-standardised and standardised methods

Cylinders reported by non-standardised/ standardised method	Mean	Standard deviation	Standard error	Correlation	p value
Hyaline casts	0,72	1,552	0,2	0,863	0,004
	1,8	4,016	0,518		
Granular casts	0,15	1,162	0,15	0,796	0,735
	0,12	0,64	0,083		

Comparison of the results obtained by both test methods revealed that the number of hyaline crystals assessed by the standardised method was statistically significantly higher that was detected by the non-standardised method (p = 0.004), and a strong correlation was observed. Increased number of hyaline casts was reported in seven samples tested by the standardised method and in only two samples tested by the non-standardised method. The KOVA method was also found to be s sensitive in detecting the presence of pathological casts in urine [10]. This result confirms that due to their consistency and shape the hyaline casts are more difficult to identify in the native (unstained) preparation. And the difference in granular cast detection by the standardised and non-standardised method was not statistically significant (p = 0.735). The slightly higher number of granular casts was detected by the non-standardised method (0.15 ± 1.162) as compared with the standardised method (0.12 ± 0.64). These casts are clearly visible regardless of the staining, and a potentially larger amount has been detected by the non-standardised method because the indeterminate amount of urine sediment was used for slide preparation.

Table 2

Comparison of epithelial cell microscopy results by non-standardised and standardised methods

Epithelial cells found by non-standardised/standardised method	Mean	Standard deviation	Standard error	Correlation	p value
Squamous epithelial cells	0,6	0,643	0,83	0,826	0,96
	0,52	0,651	0,84		
Transitional epithelial cells	0,17	0,418	0,054	0,793	0,004
	0,3	0,561	0,072		

There are recommendations developed for the correct collection of urine samples, however, these recommendations are often not followed [6]. Thus, inappropriate collection of samples leads to large amount of squamous epithelium cells present in the samples. The numbers of squamous epithelial cells in the urine sediment preparations detected by the non-standardised and standardised methods was not statistically significantly different p = 0.96

(p > 0.1). Slightly higher number of squamous epithelial cells was detected by the non-standardised method (0.6 ± 0.643) as compared with the standardised one (0.52 ± 0.651). The elevated number of squamous epithelial cells was detected in 5 samples, regardless of the test method applied. This epithelium is not difficult to identify. Higher number of transitional epithelial cells was detected by the standardized method (0.3 ± 0.561) than by the non-standardized one (0.17 ± 0.481). These cells can sometimes be misjudged as squamous epithelial cells, especially if they overlap with squamous epithelial cells. The observed differences were statistically significant (p = 0.004) and a strong correlation was observed. In order to differentiate urothelial cells of the deeper layer examination of stained sediment preparations is required. A higher number of renal epithelial cells was also detected by the standardised test (0.8 ± 0.082) as compared with the non-standardised examination method (0.22 ± 0.715). The difference was statistically significant (p = 0.008). The obtained data demonstrated that the results of our study were compliant with the results of previous research indicating that the renal epithelium was more accurately detected by the standardised method.

The increased erythrocyte levels were reported irrespective of the chemical analysis data. Our study demonstrated that a dipstick test is the more precise screening method to detect haematuria than urine microscopy or flow cytometry; it is more accurate in providing more accurate results under non-standardised test conditions [7]. A moderate corelation was reported between the number of cells detected by the non-standardised and standardised methods and the results of chemical analysis 0.591 and 0.573 respectively). It is important to note that the analyser counts not only the whole cells but also the cells affected by lysis as well as free haemoglobin and myoglobin, so a person carrying out the microscopic analysis cannot see blood in about 40 % of the examined urine samples. [8]. On the basis of these data, it can be reassured that microscopic analysis is an important part of a general urinalysis.

Table 3

Comparison of erythrocyte microscopy results obtained by the non-standardised and standardised methods

Erythrocytes detected by a non-standardised/standardised method	Mean	Standard deviation	Standard error	Correlation	p value
All samples	20,1	34,406	4,442	0,921	0,000
	36,73	58,793	7,59		
Samples that were erythrocyte - negative by chemical analysis	2,75	2,023	0,452	0,256	0,000
	6,95	3,634	0,813		

As it can be seen from the table, the samples with elevated and non-elevated erythrocyte counts were compared, although chemical analysis has not indicated any increase in erythrocyte levels (33.33 %). Comparison of the detected mean erythrocyte levels demonstrated that more cells were detected by the standardised method (36.73 ± 58.793) than by the non-standardised method (20.1 ± 34.406). This difference was statistically significant (p = 0.00). The analysis of all samples demonstrated the very strong correlation. However, a weak correlation was observed between the samples that according to European Urine Guidelines do not require microscopic analysis. The data obtained were statistically significant (p = 0.00). The number of erythrocytes detected in 17 samples examined by the non-standardised method have not exceeded the normal range. No increase was observed in four samples tested by the standardised method. The study demonstrated that a standardised method allows to differentiate erythrocytes more easily. Selection of more accurate test method improves and facilitates the process of urinary sediment microscopy.

A comparison of data obtained by the indoxyl esterase activity reaction with the results obtained by microscopic methods, demonstrated that the correlation with the standardised method was stronger (0.79)1 than that with the non-standardised method (- 0.583). Our results were consistent with the results from other studies [4].

Statistical analysis was performed with data of all collected samples (100 %) and samples for which urine chemical analysis according to CSLI is not recommended (33.33%). It was surprising that the non-standardised method detected 31.32 ± 45.46 cells on average while the standardised method reported 46.1 ± 60.136 cells. This statistically significant difference (p = 0.01), could be predetermined by the improper cell identification in the unstained preparation. Leukocyte differentiation was not carried out in the study. It is known that in some cases neutrophils may be confused with the urothelial cells of deeper layers, this may be a reason why fewer leukocytes were detected by the non-standardised examination method. The comparison

samples negative for leukocytes during the chemical analysis demonstrated a strong correlation (0.829). Standardised microscopy demonstrated more than twice higher number of leukocytes, in this case. Such test results can be explained that a known volume of stained sediment was used for examination.

Table 4

Comparison of leukocyte microscopy results obtained by non-standardised and standardised methods

Number of leukocytes detected by a non-standardised/standardised method	Mean	Standard deviation	Standard error	Correlation	p value
All samples	31,32	45,46	5,869	0,698	0,01
	46,1	60,136	7,763		
Samples negative for leukocytes during a chemical analysis	8,1	5,543	1,239	0,829	0,001
	17,25	14,98	3,35		

As the urine sediment contains no elements resembling bacteria, there are no difficulties in detecting them. Bacteria were detected in 37 samples. A nitrite index was established only in four samples and a protein index – in 32 samples. False-positive protein results can be obtained in the analyser because of leukocytes and bacteria present in the sample. Chang-Chien et al. reported in their study that proteinuria was more common in women than in men [9]. The results presented in the table show that mean bacteria count detected in all samples by the non-standardised and standardised methods of all samples was the same. The results obtained were statistically insignificant (p = 1) and also the strong correlation was reported. The samples that were negative for nitrites and protein by chemical analysis, the results obtained were not statistically significant (p = 1) and the strong correlation was reported.

Table 5

Comparison of bacterial microscopy results by non-standardised and standardised methods

Bacteria detected by a non-standardised/standardised method	Mean	Standard deviation	Standard error	Correlation	p value
All samples	0,85	0,936	0,121	0,698	1
	0,85	0,899	0,116		
Samples negative for protein and nitrite by the chemical analysis	0,25	0,444	0,099	0,808	1
	0,25	0,55	0,123		

Data presented in Table 5 show that determination of any analyte by the non-standardised or standardised method has not reached statistical significance. Sample testing has not revealed presence of pathological crystals. Increased urate levels were reported in four samples by the non-standardised method, however, no increase was detected by the standardised method. Data for uric acid crystals, triphosphates, and amorphous phosphates were evenly distributed by both methods, i.e., statistically insignificantly p = 0.027; p = 0.41; p = 1; p = 0.321, respectively.

Table 6

Comparison of crystal microscopy by non-standardised and standardised methods

Crystals detected by non-standardised/ standardised method	Mean	Standard deviation	Standard error	Correlation	p value
Urates	0,25	0,704	0,091	-0,066	0,027
	0,03	0,181	0,23		
Uric acid crystals	0,05	0,22	0,028	-0,058	0,41
	0,1	0,399	0,052		
Triphosphates	0,02	0,129	0,017	-0,017	1
	0,02	0,129	0,017		
Amorphous phosphates	0,12	0,524	0,068	0,87	0,321
	0,08	0,462	0,06		

The table below illustrates the additional elements that were detected found during the study. The study results are characterised by almost evenly distribution. Significant increase in mucus was not reported in any of the samples. Higher level of mucus was detected by the non-standardised method (0.22 ± 0.454) as compared with the standardised method (0.17 ± 0.376). This statistically insignificant difference may have been predetermined by the inaccurate amount of urine sediment used for the slide preparation, as the larger sample volume may contain, larger amount of mucus. Detection of fungal spores by both methods was not statistically significant, however, the standardised method was associated with slightly higher amount of detected fungal spored (0.03 ± 0.181). Some spores could be confused with erythrocytes in a non-standardised assay (0.02 ± 0.129). Sperm detection was similar in both methods.

Table 7

Comparison of microscopy results of other elements by non-standardised and standardised methods

Non-standardised/standardised method found	Mean	Standard deviation	Standard error	Correlation	p value
Mucus	0,22	0,454	0,059	0,579	0,321
	0,17	0,376	0,049		
Spores	0,02	0,129	0,017	0,701	0,321
	0,03	0,181	0,023		
Sperm	0,05	0,22	0,028	-	-
	0,05	0,22	0,028		

Microscopic analysis of samples that did not show any chemical abnormalities revealed that the majority of mismatch cases were found between erythrocyte and leukocyte analytes. An analysis of these data revealed a marked difference between the standardised and non-standardised test method. The standardised method, was associated with the higher numbers of detected cells and more frequent deviations from normal values as compared with a non-standardised method (Tables 3 and 4). These samples did not contain transitional epithelium. Though precipitate staining does not affect crystal detection, an increased crystal levels were reported in three samples tested by a standardised method. Deviations of Renal epithelium were reported by a standardised method only, and the increase in squamous epithelium levels was thought to be related to incorrect sample collection. Bacteria were detected in the majority of samples irrespective of the test method.

Conclusions

1. According scientific research, the most accurate results are obtained by the microscopy of stained urine sediment applying a standardised method. Microscopic analysis can also be carried out by an automated flow cytometry.

2. Out of 20 urine samples in which the increase in erythrocyte and/or leukocyte count and/or protein levels during chemical analysis was not reported, deviations from normal values were reported in 17 samples tested by standardised and non-standardised microscopy in methods and only 3 samples were reported as having no changes. An increase in erythrocyte and leukocyte levels was detected in the majority of samples, with a statistically significant difference between the methods (p = 0.00 and p = 0.01). Bacteria were detected in the majority of samples irrespective of the method. Renal epithelium was detected by a standardised method only. The increase in squamous epithelium levels in these samples was not significant and was associated with poor compliance with sample collection requirements. It can be suggested that urine chemical analysis not including sediment microscopy is not a reliable method if used as the only diagnostic method.

3. Statistically significant difference was reported between the results of standardised and non-standardised urine sediment test of hyaline casts (p = 0.004), erythrocytes (p = 0.000), leukocytes (p = 0.01), transitional epithelial cells (p = 0.004) and renal epithelium cells (p = 0.008). No statistically significant difference was found between granular casts (p = 0.735), squamous epithelium cells (p = 0.096), bacteria (p = 1), urate (p = 0.27), uric acid crystals (p = 0.41), triphosphate (p = 1), amorphous phosphates (p = 0.321). On the basis of our study results it could be concluded that the standardised test method is a more reliable and expedient option than the non-standardised method.

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