

Practical application and evaluation of usage of devices for biochemical and cytological urinary status

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ABSTRACT

Aim To explore advantages and disadvantages of visual and automated dipstick reading for biochemical urine analysis, and to compare classical methods (reaction of protein confirmation, examination of urinary sediment) with dipstick analysis.

Methods Testing was conducted as a prospective study in the Institute of Occupational Health of Zenica-Doboj Canton, Bosnia and Herzegovina. Urine samples were collected during the period of three months (from February till May 2012) from two groups of patients: 100 urine samples from healthy people (annual health screenings) and 100 urine samples from patients with urinary tract disorder. Visual and automated reading of two devices (Meditron Junior II Roche and Mission U120 Urinalysator ACON Lab. Inc.) and two kinds of dipsticks were compared.

Results The Mission U120 showed statistically significant lower levels of leukocytes and erythrocytes in urine compared to Meditron Junior II ($p=0.0005$ and $p=0.005$, respectively), and cytological control was crucial for these estimates. In the first group of healthy people proteins in urine were found in 76 out of 100 cases (76%) in automated reading. This was not shown in visual reading of test stripes (20% sulphosalicylic acid test was negative). In the second group of patients visual and automated reading of dipsticks for nitrites was not followed by cytological findings.

Conclusion Test strips application should be monitored considering their false positive and false negative results. Devices for automated test strips reading are important part of modern laboratory. Due to disadvantage of classical urinary sediment analysis the aim should be focused on devices for completely automated urine analysis.

Key words: dipsticks, urine, analyzers.

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INTRODUCTION

Urinary system regulates volume and composition of body fluids in a way that kidneys eliminate toxic substances from blood plasma producing urine (1). Urinary test results are composed of markers of physical characteristics of urine, markers of chemical characteristics of urine, and urine sediment (2). Examination of urinary test results gives a lot of data that are significant for diagnosis of numerous renal and extrarenal disorders (2).

Physical characteristics of urine are urine leer, color, smell and specific weight (3). Leer of fresh normal urine is completely clear and transparent. Pathological abstruseness is caused by various salts, cellular elements, mucus, epithelial cells and microorganisms (2). Normal urine has a from pallid to yellow-orange color combinations (1). Causes of urine color changes can be various (3), such as erythrocytes present in urine (red color), hemoglobin present in urine (red-brown color), bilirubin present in urine (urine color is from dark yellow to brown). Normal fresh urine has characteristic smell that is similar to one from bovine soup (3). Urine specific weight is defined as weight of urine volume in comparison with the same volume of distilled water, and is in interval from 1.005 and 1.030 (3, 4).

Chemical urine examination including other various methods serves for verification of normal and pathological urine compounds. Urinary test strips are used for this purpose nowadays, and further markers of chemical characteristics of urine are identified: pH value, proteins, blood, leukocytes, carbohydrates, ketones, nitrites, bilirubin and urobilinogen (2). Urinary pH value is between 5 and 9, and normal finding is between 5 and 6 (1). Proteins are not present in normal urine. Golden standard for confirmation of proteinal urinary presence is 20% sulphosalicylic acid (5). Blood is also not present in urine of healthy persons. Normal finding in urine sediment is up to 5 erythrocytes (6,7). Leukocytes finding in urine of healthy persons is negative. Normal finding in urine sediment is up to 12 leukocytes (1). Tests for glucose determination are used for diagnosis and control of metabolism of carbohydrates. Glycosuria was not present in healthy persons (1). Ketones are intermediate products of metabolism of fats that are excreted in urine. Ketonuria is present in diabetes mellitus and starving (1). Appearance of nitrites in

fresh urine is always a sign of urogenital tract infection. In this case there has to be enough concentration of nitrates in urine as a substrate for the reaction to take place (3). Bilirubin and urobilinogen are important in examination of hepatobiliary system, and they can not be found in urine of healthy persons (1).

For routine urine analysis first urine in the morning has to be taken. Urine sample has to be collected in one-time plastic sterile, or chemically disinfected containers. Minimal volume sufficient for analysis is 10 ml of urine. Urine analysis has to take place not later than 2 hours from its collection (4).

Test strips are multilayer plastic strips and they are produced for visual and automated biochemical urine analysis. They contain chemically impregnated fields with absorbing plumper. These fields are composed of foil carrier, absorbing paper, paper with specific reagent and nylon reticule, and they are specific for the component of urine which is analyzed (pH value, proteins for example). Chemical reaction between the examined component and impregnated fields is seen as specific color change. Intensity of the color is in proportion with examined urinary component. There are various test strips with different numbers of impregnated fields on the market, depending on devices for the automated test strips reading (6,7).

Test strips analysis is manual (visual) and automated (with devices). Visual test strips reading is done after certain period of time from immersing the dipstick into examined urinary sample by the person who makes analysis. Disadvantages of visual test strips analysis are that this kind of analysis cannot be standardized, due to different capability of person that performs analysis, due to different environmental conditions that can influence proper results readings, etc (6,7).

The function of the devices for automated test strips reading is based on principles of reflexive photometry (1). Systems for automated test strips reading are divided into three groups (1): instruments for single readings (that measure intensity and color of the dipstick for urinary analysis) (1,8), semi-automated systems for urinary analyses (1,9), and fully automated systems for urine analysis (which have two parts, one for test strips reading and the other for microscopic examination of urinary sediment (1,10).

Urinary sediment is a precipitate that arises

after urinary centrifugation. It serves for microscopic examination that has great significance for diagnostics and monitoring of renal disorders. Sediment is composed of organized and unorganized part. Unorganized part contains various salts that appear in crystal or amorphous shape. Organized part contains epithelial cells (squamous epithelium), single leukocytes, microorganisms or random components (4).

The aim of this study was to explore advantages and disadvantages of visual and automated test strips reading for biochemical urine analysis, to compare classical methods (reaction of protein confirmation, examination of urinary sediment) with test strips analysis.

MATERIALS AND METHODS

Testing was conducted as a prospective study in the Department of Laboratory Diagnostics, Institute for Occupational Health of Zenica-Doboj Canton. Urine samples were collected during the period of three months (from February till May 2012) from two groups of patients: 100 urine samples from healthy people (annual health screenings) (I group) and 100 urine samples from patients with urinary tract disorder (II group).

The research was approved by the Ethical Committee of Institute of Occupational Health of

Table 1. Statistically significant differences between visual and automated test strips reading in group I patients

	I group (healthy patients)			
	TTMID	MID	TTMISS	MISS
Specific urinary weight				TTMID
				MID
			Z=-3.947 p<0.0005	TTMISS
		Z=-3.829 p<0.0005		MISS
Urinary leukocytes				TTMID
	Z=-4.804 p<0.0005		Z=-4.116 p<0.0005	MID
			Z=-3.051 p<0.005	TTMISS
				MISS
Urinary pH value				TTMID
	Z=-4.115 p<0.0005			MID
				TTMISS
		Z=-5.329 p<0.0005		MISS

TTMID, visual reading of test strip for Miditron Junior II; MID, Miditron Junior II; TTMISS, visual reading of test strip for Mission U 120; MISS, Mission U 120

Zenica-Doboj Canton.

Research methods were cytological and microbiological. Performed biochemical analyses were used to compare visual and automated test strips readings. It compared visual test strips reading (with two types of dipsticks of different manufacturers) and automated reading of two devices (single-reading instrument Mission U120 Urinanalysator /ACON Lab. Inc., USA/ and semi-automated instrument Miditron Junior II /Roche Diagnostics, Switzerland/) (8,9).

Cytological analyses implied microscopic examination of urine sediment. In cases where bacterial infection was suspected (nitrites found in urine) microbiological analysis was conducted.

Results were presented in form of descriptive statistics, and statistical analyses were performed by non-parametric *Wilcoxon* signed-rank test for related samples with level of significance $p<0.05$.

RESULTS

The results of analysis of specific urinary weight, urinary pH value, number of erythrocytes and leukocytes have shown statistically significant differences in reading of two types of test strips for Mission U 120 and Miditron Junior II ($Z=-3.829$, $p=0.0005$; $Z=-5.329$, $p=0.0005$; $Z=-2.985$, $p=0.003$; and $Z=-7.198$, $p=0.0005$, respectively). The results were similar between visual and automated reading of test strips regarding these components ($Z=-3.051$, $p=0.002$; $Z=-2.887$, $p=0.004$, $Z=-3.573$ $p=0.0005$; and $Z=-4.253$, $p=0.005$; respectively), and also between automated reading of the instruments themselves ($Z=-3.043$, $p=0.003$; $Z=-3.947$, $p=0.0005$; $Z=-4.184$, $p=0.005$; and $Z=-3.998$, $p=0.005$; respectively). For the rest of the components glucose, acetone, bilirubin, urobilinogen and nitrites there were no statistically significant differences between visual and automated reading of test strips ($Z=-0.255$, $p=0.98$; $Z=-1.224$, $p=0.23$; $Z=-0.423$, $p=0.67$; $Z=-0.697$, $p=0.49$; and $Z=-0.435$, $p=0.65$; respectively). Statistically significant differences were obtained in the first and also in the second group of patients (Table 1, Table 2).

In the first group of patients the Mission U120 apparatus showed statistically significantly lower levels of leukocytes and erythrocytes in urine compared to Miditron Junior II

Table 2. Statistically significant differences between visual and automated test strips reading in group II patients

	II group (patients with disorder)			
	TTMID	MID	TTMISS	MISS
Specific urinary weight				TTMID
				MID
				TTMISS
		Z=-3.844 p<0.0005		MISS
Urinary leukocytes				TTMID
				MID
				TTMISS
	Z=-3.573 p<0.0005		Z=-2.887 p<0.005	MISS

TTMID, visual reading of test strip for Miditron Junior II; MID, Miditron Junior II; TTMISS, visual reading of test strip for Mission U 120; MISS, Mission U 120

($p=0.0005$ and $p=0.005$, respectively). Both of these devices showed significantly higher values than those found in urinary sediment, and cytological control that was crucial for these estimations.

The comparison of classical and test strips reading has shown statistically significant differences between leukocytes and erythrocytes values of healthy and patients with urinary problems ($p<0.0005$ and $p<0.0005$, respectively).

In the first group of healthy people proteins in urine were found in 76 patients (76%) in automated reading. This was not shown in visual reading of test strips in any of the patients, and also 20% sulphosalicylic acid test was negative ($Z=-6.633$, $p=0.0005$). In the second group of patients visual and automated reading of test strips for nitrites (found negative) were not followed by cytological findings (bacteria found in urine). Inverse cases were rare. In dozen of cases findings of bacteria in urine sediment were not followed by microbiological findings (bacterial flora that was not pathogenic or physiologic flora at all) ($Z=-3.667$, $p=0.0005$).

DISCUSSION

Biochemical and cytological urine status is a very important analysis that cannot be ignored, because the results can warn about some pathological changes in organism during early stages. That is why it is important for all laboratory personnel to understand and maintain basic working principles in order to achieve most accurate and precise results. Any kind of mistake in this part can lead to mistaken labo-

ratory findings as a consequence (11,12).

In many laboratories test strips for visual urine analysis are used, while there is mistrust to devices for automated urine analysis without any reason (13). Instruments usage facilitates laboratory work and increases personnel productivity (11,12). The implementation of urine analyzers was the main reason for great improvements in the Department of Laboratory Diagnostics that were achieved over the years, which were accomplished with careful handling and respecting the possibilities of devices for automated test strip reading. These facts were the reason why this study was initiated.

It is a well-known fact that devices for automated urine analysis are more precise than visual abilities of a human eye. That is why the focus should not be on the question whether to use devices or not, but whether we can trust the dipsticks or not. If the answer to this is affirmative, then the next question is what kind of dipsticks we have to trust, to what extent and in which cases (11,12,14). Problems can be caused by test strips with expired validity period, those inadequately stored, strips exposed to air or some kind of contamination (6,7). In these situations human factor is also unavoidable (possible displacement of samples, test strip fields in visual reading can be mixed up in personnel's memory and interpretation, wrong interpretation of color intensity in the test strip fields, flaws in immersing the dipsticks completely into urine, urine storage before analysis /no longer than two hours/, urine drops over the dipstick /false positive urinary nitrites findings, adequacy of containers for urine sampling, purity of other laboratory equipment, etc.) (1). All of this could lead to false positive or false negative results (6, 7). For the screening test it is always a bigger problem to get false negative results than false positive results, because in routine daily work there are no obvious reasons to check negative results (1). Because of that it is important to observe the urine in the container (tube), because its leer is obvious (muddy, fetid), and also to compare the urine pathology with other biochemical findings (2). In that way it would be confirmed if the expected results were obtained, and in suspicious cases analyses using classical methods have to be repeated (11,12,14). Due to this it was important to emphasize that this study indicated all of these moments through the importance of combination of automated urine analysis with cytological examination of

urinary sediment.

Results obtained in the study showed that devices and strips represented higher values of leukocytes, erythrocytes and proteins than the classical methods. That is why it was decided to show findings of pathological urine with cytological findings (microscope) and with confirmation with classical method of sulphosalicylic acid reaction. This was made in order avoid confusion of therapists who are guided with old habits and attitudes, and fail to inquire whether referent values have been changed, which is often the case with the new instruments. These are the only significant differences between classical methods and test strips. Zamanzad claims that dipstick urinalysis can be a reliable screening method for diagnosis of urinary tract infection and diabetes mellitus but not for proteinuria (12). Urine examination does not mean only automated urine analysis, but also combination of this with sediment analysis (1-5). This is exactly that was shown in this study in the first group of examined samples for leukocytes and erythrocytes values in relation to cytological analysis of urinary sediment.

Analysis of cytological urinary findings in this study showed that in signs of urinary tract infection only in 50% of cases a cause of infection was isolated. In other 50% cases it was found that pathogenic bacteria were not isolated, or that urine was sterile. This information led to the conclusion that when microscopic sediment analysis showed information about many bacteria present in it, this could not nec-

essarily mean urinary infection, but that the reason for this could be nonpathogenic bacteria, some decomposed artifacts occurred after centrifugation or some other contamination (15,16). This is the reason why biochemical laboratory should aspire to new generation of devices for completely automated urine analysis together with sediment examination with camera and final image of urinary sediment (17,18). In this way decomposition of elements is avoided because there is no centrifugation, and this is of great help to the laboratory personnel and the therapists. The devices of the new generation are not available to us yet due to their high price. There are numerous arguments that these instruments would bring us the great improvement in urinary sediment analysis area, which is currently at the level of microscopic examination (17,18).

In conclusion, devices for automated test strips reading are important part of modern laboratory. Due to disadvantages of classical urinary sediment analysis the goal should be to get devices for completely automated urine analysis. In biochemical urine analysis human factor is very important, in terms of decreasing their own mistakes, but also in terms of daily control of the findings produced by the instruments.

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TRANSPARENCY DECLARATIONS

Competing interests: none to declare.

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Praktična primjena i procjena upotrebljivosti aparata za biohemijsko citološki status urina

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SAŽETAK

Cilj Istražiti prednosti i nedostatke vizuelnog i automatskog očitavanja testne trake kod biohemijske analize mokraće, te uporediti klasične metode (potvrдна reakcija za proteine, pregled sedimenta mokraće) i analizu s testnim trakama.

Metode Ispitivanje je provedeno kao prospektivna studija na Zavodu za medicinu rada Zeničko-dobojskog kantona u Zenici (Bosna i Hercegovina). Uzorci mokraće prikupljeni su tokom tromjesečnog perioda (od februara do maja 2012. godine) od dvije grupe pacijenata: 100 uzoraka mokraće od zdravih osoba (godišnji kontrolni pregledi) i 100 uzoraka od pacijenata sa zdravstvenim problemima u mokraćnom traktu. Poređeno je vizuelno i automatsko očitavanje dvije vrste aparata (*Miditron Junior II Roche* i *Mission U120 Urinalysator ACON Lab. Inc.*) i dvije vrste testnih traka.

Rezultati Aparat *Mission U120* pokazivao je statistički značajno niže vrijednosti leukocita i eritrocita u mokraći u poređenju s aparatom *Miditron Junior II* ($p=0,0005$ i $p=0,005$, respektivno), dok je citološka analiza bila od presudne važnosti za ove procjene. U prvoj grupi zdravih osoba proteini u mokraći su nađeni u 76 od 100 slučajeva (76%) kod automatskog očitavanja. Ovo nije pokazano pri vizuelnom očitavanju testnih traka (test s 20% sulfosalicilnom kiselinom bio je negativan). U drugoj grupi pacijenata, vizuelno i automatsko očitavanje testnih traka kod analize nitrita u urinu nije bilo praćeno s citološkim nalazima.

Zaključak Upotreba testnih traka treba biti pod nadzorom s obzirom na lažno pozitivne i lažno negativne rezultate. Aparati za automatsko očitavanje testnih traka važan su dio moderne laboratorije. S obzirom na nedostatak klasične analize sedimenta mokraće treba ciljati na aparate za kompletno automatizovanu analizu mokraće.

Ključne riječi: testne trake, mokraća, analizator.