

## Evaluation of Urine Analysis in Addiction Wards in a Clinical Laboratory

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### ABSTRACT

**Aim:** sixty urine samples of abusers were examined for macroscopic and microscopic process. Urine sediments and their contents were directly evaluated with microscope. All of the study population were male and their age range were (Mean  $\pm$ SD) = (40 $\pm$ 22). All study group filled satisfy form to participating in study. At the same time a macroscopic procedure for the screening other urine parameters have been developed.

**Method:** Urine samples were daily collected from abusers of Tabriz, Iran. Urine analysis were prepared using macroscopic and microscopic methods. The sediments were made by centrifuging 4000rpm for 10 minutes. After separation of sediments, one droplet of the residue was evaluated by a lam and lamel and a microscope. For screening, all samples were directly exposed with urine analysis rapid strip dipsticks to determine some urine parameters.

**Result:** The data analyzed by SPSS and ANOVA one way. The result of this study has shown that approximately all of urine specimen from abusers in welfare organization of Tabriz /Iran had contained drug casts or drug crystals in urine sediments.

**Conclusion:** This study describes importance of urine analysis of abusers in their detoxification term.

**Key words:** Practical performance, urine analysis, rapid dip stick test, macroscopic, microscopic, Urine particles

### Introduction

Several studies have performed on biological specimen to diagnosis of illness through humans' body fluids. Hippocrates described renal failure and chronic diseases by the appearance of hematuria and bubbles on the urine samples surface. Urinalysis is one of the most first basic practical tests to the presence, severity, and diseases of the kidney and urinary tract [7]. For the clinicians and nephrolo-

gists automated urine technology and other centralized laboratory testing provide standard data of urinalysis [1,9,12,13]. Urine can be collected routinely in high quantities and will not cause damage to the human body organs. Urine is the normal metabolic fluid of the creators, and in the normal condition urine will not have an adverse effect on the human's body. According to database of UMDB

(Urine Metabolome Data), metabolites of urine specimen have been found above 220 diseases. However deep stick urine tests are used to assess whether there has been recent alcohol and other drug abuse consumption. The rule of monitor, whether the urine contains human chorionic gonadotropin hormone (HCG). Then urine can be used to examine whether women are pregnant or not, and the same time the results can be achievements within fast as a few minutes. However, at present in the study of urine biomarkers importance, urine biomarkers been continuously found in lung cancer, cardiovascular disease, blood coagulation system, renal cancer and other kidney diseases, bladder cancer, prostate cancer, Alzheimer's disease and autoimmune diseases and other malignant tumors [4]. Some studies have performed on humans' body fluids to determine drugs or other biomarkers. It is of increasing concern that abusers involved in abuse drugs are exposed to these compounds which usually causes different body organs disorder or diseases. The most requested routine tests are the general urine analysis, which covers of biochemistry analysis (pH, glucose, urobilinogen, bilirubin, ketone, acids, protein, blood, nitrite, specific gravity, etc.), and physical appearance (color, odor aspects). The light microscopic analysis of urinary sediment in search of formed parameters (yeasts, crystals, mucus, erythrocytes, urothelial cells leukocytes, bacteria, casts, etc.). Urine examination has essential role in patients with various diseases and provides the physician with very important data to support the diagnosis and management of several renal pathologies assessments. So, several studies including urine analytical tests in clinical and forensic laboratories for drugs diagnosis support in urine sediments have been developed. With this background we carried out evaluation of urine analysis of abusers at clinical laboratory under vision of welfare organization and Hygiene centrals of Tabriz, Iran [13].

## Materials and Methods

### Urine specimen descriptive

Not only a correct urinalysis offers a direct indication of the state of the patient's kidney and genitourinary system but also a monitor of the other body systems. Afterwards the complete examination has been divided two pathway evaluation of macroscopic and microscopic in urine specimen. The macroscopic analysis of urine includes assessment of its physical appearance characteristics (odor, specific gravity, temperature, and color) and chemical analysis. Microscopic analysis of the constituents of urine is performed more usually, the sediment from a centrifuged tube button section of urine samples.3. A droplet of the urine sediment residue was transferred onto a glass lam for evaluation. The sediment was first examined under low power to identify most crystals, casts, renal and urinary tract cells and other large particles as and bacteria, RBC, yeasts... Since the number of elements found in each field may vary considerably from one field to other fields, thus all fields were averaged. Then examination was carried out at high power to identify large elements as WBC, yeasts crystals, epithelial cells and casts. Finally, the different types of renal cells were described as the number of each type found per average high power field under 40 number lenz of light microscope. (HPF) [eg: 1–2 RBC/HPF]. AMU = amorphous urate crystals; AU= Ammonium urate; AMP = amorphous phosphate crystals; CC=cysteine crystals UA

= uric acid crystals; MS=Magnesium sulphate; COA = calcium oxalate crystals; SUL = sulphates; TP = triple phosphate crystals; DC=drugs crystals. Though the urine centrifuged method had a valuable identification level for the urine sediments examined. Then, when combined with urine analytical biochemistry and microscopic evaluation, both tools should provide higher information as a screening method in routine urine practical analysis [3]. Sediment analysis are frequently performed in the urology outpatient department, as well as in other departments and general practices. All urine samples from a population of patients visiting the urology specialist physicians were analyzed [6].

according to update recent studies evaluated automated urine analyzers, the range of values used was very limited by the fluorescence flow cytometry method to recognize and classify 17 types of urinary parameters such: Red blood cells, Non lysed RBC, White Blood Cells ,WBC clumps, Total Epithelial Cells, on-Squamous Epithelial Cells , Squamous Epithelial Cells, Transitional Epithelial Cells, Renal Tubular Epithelial Cells, CAST, Hyaline CAST, Pathological CAST, Bacteria, Crystals, Yeast-like cells , Spermatozoa and Mucus [5,13-20].

## Collection of Specimens

### Physical Parameters

First morning urine samples were provided with 100ml volume for every test.

### Color in urine

Appearance of Specimens might be: hematuria, hemoglobinuria, myoglobinuria (pink Color, red Color, brown Color, black Color), liver disorder Jaundice (yellow to brown Color), chyluria (white fat milky urine), massive uric acid crystaluria (pink Color) porphyria, alkaptonuria (red to black color after standing) and so on.

### Chemical Parameters

pH in practice, pH is routinely measured with the help by dip stick method. This is based on an indicator which covers the pH normal range 5.0 to 8.5 to 9.0. With this method, then significant deviations from true pH can be observed for values range less than 5.5 and higher than 7.5. However, a pH meter with a high sensitivity glass electrode is requirement when accurate measurement in research laboratories. A part from above mentioned in clinical practice, measurement of urine pH is necessary for correct description of urinary microscopic findings. Hence, intake of proteins and acidic fruits (e.g., Lemon) can result in acidic urine [7]. Renal tubular acidosis or alkalosis change urine pH that led to urinary tract crystals uric acid calculi can be find in acidic urine, Alkaline urine can be associated with magnesium ammonium phosphate crystals or alkaline structure drugs or abuse compounds and make stag horn calculi. (e.g. Crystal= X'TAL) [7].

### Drugs

Drugs may be changing urine pH and appearance odor and color: Rifampin, Vitamin B2, phenazopyridine, isoniazid (yellow-orange to red color of urine) phenytoin (red color), chloroquine, nitrofurantoin (brown color), triamterene, blue dyes administered by

feeding tube. (Green color) metronidazole, Methyldopa, imipenem, cilastatin (darkening of urine upon standing [7]. Moreover some of abuse drugs might be intake by abusers of this study group were: Heroin, Morphine, Cannabis, Methadone, Amphetamine, Methamphetamine, Methylene Dioxy methamphetamine, Cocaine, Phencyclidine, and other medications were: Three cyclic antidepressant, Benzodiazepine Tramadol, Buprexin, adult cold, chlorodizpoxide, natrium voltaren, antihistamine deconjuant, amitriptyline, acetaminophen codeine, imipramine, flouxetine, perphenazine, diazepam, atenolol, phenobarbital, theophylline, pantazosine, Amoxicillin, cimetidine, difenoxilate, Indral, spironolactone, caffeine, phenilbutazone, Antibiotics, vitamin minerals too. All of above-mentioned compounds have alkaline structure and will gave proper reaction in  $pK_b > 7$ . So, for pH improvement before doing test one droplet amoniaque was added to every urine sample [10]. The brand rapid dip stick test used in this study are commercially available by Iranian or foreign products [13].

Selection of study group: All 60 abusers from central campus taking part in this study received detailed information documented by morality ethical committee of the medical sciences university and filled in consent form concerning of the research study aims [13].

## **Samples providing**

### **Sampling**

Overall, 60 urine samples were collected in study duration. All of participants were male mean aged were  $40 \pm 22$  years. All of them were smokers and had drug consumption depends on themselves medications. All gave their informed consent prior to their in the study. All of urine samples were daily collected from abusers and saved in refrigerator, then pH of urine samples evaluated. The pH regulated with pH paper for each sample. Primary samples of all volunteers obtained from morning urine were transferred to laboratory in capped sterile beakers. The samples were maintained in refrigerator for investigation of microscopic urinary sediments.

### **Reagents**

All chemicals used in this study were of analytical grade and obtained from (Merck co, Germany). All medication compounds of clinics purchased from Zahravi pharmaceutical valuable company, Tabriz, Iran [13]. 100ml of urine samples were received from every patient for urine analyzed process (U/A). First some of samples were directly examined for presence or absence of some urine parameters with rapid strip dip stick tests. Each addicted subject was asked to give a first morning fresh sample of his urine to the laboratory before treatment.

### **Preparation of urinary sediments**

All the urine samples from the patients were centrifuged for 8 minutes using large falcon tubes (4000g). A mixture of  $20 \mu\text{l}$  of urinary sediments evaluated by Zeiss 20 X of 40 microscope lens. Under microscope, parameters (yeasts, crystals, mucus, erythrocytes, urothelial cells leukocytes, bacteria, casts, etc.). Also Death and vital cells can be distinguished by blue and lemon-yellow cytoplasm respectively. Other components of urinary sediments

such as urinary casts, mucus, calcium oxalate crystals, uric acid and urates amorph and drug crystals were studied [13]. Urine sediment is analyzed for various elements, which contain to cells, mucus, casts, microbes, crystals... casts are cylindrical markers that formed in the collecting ducts or distal tubules and, they might be hyaline or acellular, include granular or waxy material, or various cell types (erythrocytes, leukocytes, and RTECs). Crystals are formed in urinary tracts by oversaturation of substances that produced through metabolic and inherited diseases or other drug overdose exposures. However, facilitates of crystal formation and information of accurate characterization is dependent to urine pH. [1,8-13].

### **Statistical analysis**

The statistical analysis of data was done with SPSS followed and was carried out and using ANOVA one way. The level of significance was chosen at  $p < 0.05$  [10,12,13].

## **Results**

As limitation of the process of urine specimens' collection, as stated in this method, we used primary morning urine samples. Morning urine specimens were collected in a sterile container for urine sediment analyses [6]. All of the abusers had conditions history of smoking, alcohol, pain relief and sedative medications. Majority of the addicts had relatively long history of using addictive substances or drugs for more than five years. Some clinical laboratory tests were conducted on the biological fluids samples and then their results were recorded. Moreover, some studies were also carried out to investigate infectious level of the abusers. First, all urine samples were examined with rapid dip stick tests then all urine sediments evaluated through light microscope analysis method. However, the results of urine strip test for drug abusers while they were positive under medication. Because abusers take some other medication under their physicians' orders, then their results might be explaining drugs crystals in urine sediments. All calculation were performed using SPSS software. The data were examined by using of variance (ANOVA ONE WAY) by post hoc comparison between groups of student Newman kelus test. Differences in which  $p < 0.05$  were considered statistically significant. The results of this study show that above 50 percent of urine samples from abusers in Tabriz, Iran were contained crystals and abuse drug crystals in urine samples [10,12,13,14,15]. According to this research database all tables describe: Table 1: Shows number of some of the drugs and abuse drugs along with directly rapid strip tested between the study populations. Table 2: Some of Urine sediments results during the period of medication in population study groups. Table 3: Some of Urine sediments major abnormal parameters during the period of microscopic urine analysis in population study of groups. Table 4: Some of Urine sediments major abnormal parameters during the period of microscopic urine analysis in population study groups. Table 5: Some of Urine major abnormal parameters during the period of macroscopic urine analysis in population study groups. Figure1: Show some crystals in urine sediments of study group.

**Table 1:** Shows number of some of the drugs and abuse drugs along with directly rapid strip tested between the study population.

No	Drug names	Men	Abuse Consumption history	patients' numbers
	Drugs Medication	Ages Mean Mean±SD	year	N
1	Flouxetine	55	2y	N=60
2	Voltaren	44	3y	N=60
3	Propranolol	41	2y	N=60
4	Methadone	40	1y	N=60
5	THC	40	4y	N=60
6	Bupernorphine	30	1y	N=60
7	Codein	60	5y	N=60
8	Paracetamol	59	5y	N=60
9	BNZ	32	1y	N=60
10	TCA	31	2y	N=60
11	BAR	35	1y	N=60
12	Theophiline	58	4y	N=60
13	TRM	60	6y	N=60
14	Caffeine	61	4y	N=60
	Antihista-mines			

Ages mean>21, N=60, BNZ: Bezodiazepines, TCA: Three cyclic antidepressants, BAR: Barbiturates, TRM: Tramadol.

**Table 2:** Some of Urine sediments results during the period of medication in population study groups.

No	Men	Consumption history	Before detoxification	After detoxification
	Age	month or year	Drugs X'TAL	Drugs X'TAL
1	61	21y	±	-
2	47	13y	+	±
3	46	12y	+	-
4	42	10y	+	-
5	39	8y	+	-
6	33	8y	+	-
7	39	9y	+	-
8	38	7y	+	-
9	35	7y	+	-
10	34	5y	±	-
11	35	3y	+	-
12	36	6y	+	-
13	47	10y	+	-
14	41	9y	±	-
15	43	6y	+	-
16	41	10y	+	-
17	44	13y	+	-
18	51	17y	+	+
19	50	9y	±	-

20	38	8y	+	-
21	40	6y	+	-
22	37	4y	+	-
23	34	8y	+	-
24	39	11y	+	-
25	58	9y	±	-
26	41	12y	+	-
27	39	4y	+	-
28	31	6y	+	-
29	30	15y	+	±
30	60	35y	±	-

Abuse drugs (Heroin, opium, Methadone, Codeine, cannabis, Methamphetamine) detection in abusers men Urine sediments. Medication (TCA, BNZ, BAR, TRM...) BNZ: Benzodiazepines, TCA: Three cyclic antidepressants, BAR: Barbiturates, TRM: Tramadol. ±: weak counts of X'TALs: Crystals

Statistical analysis: ANOVA one way. The level of significance:  $p<0.04$ .

**Table 3:** Some of Urine sediments major abnormal parameters during the period of microscopic urine analysis in population study of men groups.

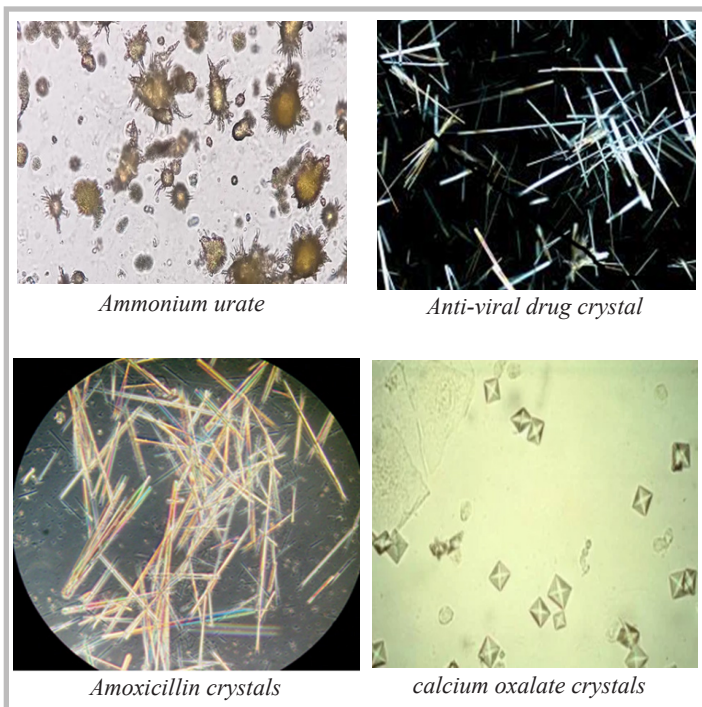
No	Men	Casts	Cells	Red Blood cells	Microbes Bacteria	Crystal	Leuco-cytes
	Age	Casts	Epithelial Cells	RBC	Bacteria	X'TAL	WBC
1	24		21	1	Many	-	11
2	38		4	1	-	-	6
3	26		3	1	Mod	-	17
4	31		27	6	Many	-	11
5	25		29	28	Many	-	29
6	26		3	1	Mod	-	7
7	38		4	1	-	-	21
8	36		27	3	Many	-	14
9	34		22	1	Many	-	29
10	33		2	2	Mod	-	6
11	29		3	1	-	-	7
12	34		2	1	-	±	9
13	25		21	3	Many	-	8
14	21		9	2	Mod	-	5
15	23		8	2	Few	+	4
16	42		5	3	Few	±	2
17	45		4	3	-	-	1
18	53		6	4	Mod	+	3
19	57		2	2	Many	-	8
20	61		11	3	Many	+	9

The numbers of cells (Epithelial cells, white Blood cells=WBC, Red Blood Cells=RBC) in per microscopic field. (± : Trace amount).

The numbers of cells (Epithelial cells, white Blood cells=WBC, Red Blood Cells=RBC) in per microscopic field. (± : Trace amount). .( ±: weak counts of X'TALs: Crystals).



**Figure 1:** some crystals in urine sediments of study group.



**Table 4:** Some of Urine sediments major abnormal parameters during the period of microscopic urine analysis in population study groups.

No	Men	Casts	Mucus	Crystal
	Age	Casts	Mucus	X*TAL
1	24	+	++	-
2	38	-	-	-
3	26	-	-	-
4	31	-	-	-
5	25	-	-	-
6	26	-	-	-
7	38	-	-	-
8	36	-	-	-
9	34	-	-	-
10	33	-	-	-
11	29	-	-	-
12	34	-	-	±
13	25	-	-	-
14	21	-	+	-
15	23	-	+	+
16	42	-	-	±
17	45	-	-	-
18	53	+	+	+
19	57	-	++	-
20	61	+	+	+

Calcium oxalate crystals, Uric acid crystals and acidic drugs crystals were often seen in acidic urine pH ( $pH < 7$ ). Abuse drugs crystals were seen in some urine pH variations. Hyaline casts were seen in urine sediments in some cases. (±: weak counts of X\*TALs: Crystals).

**Table 5:** Some of Urine major abnormal parameters during the period of macroscopic urine analysis in population study groups.

No	Men	position	pH	Protein	Glucose	Nitrite
	Age	Color	pH	Protein	Glucose	Nitrite
1	24	Ysc	5	+	-	-
2	38	Ysc	5	-	-	-
3	26	Ysc	5	-	-	-
4	31	Ysc	6	-	-	+
5	25	Ysc	6	-	-	-
6	26	Ysc	5	-	-	-
7	38	Ysc	5	-	-	-
8	36	Ysc	5	-	-	-
9	36	Ysc	5	-	-	-
10	34	Ysc	5	-	-	-
11	33	Ysc	5	-	-	-
12	34	Ysc	7	-	-	-
13	25	Ysc	6	-	-	-
14	21	Y	6	±	-	-
15	23	Ysc	5	-	-	-
16	42	Yc	5	-	+	-
17	45	Yc	5	-	-	-
18	53	Ysc	6	+	-	-
19	57	Yt	7	+	+	+
20	61	Ysc	6	+	-	-

Urin es color status (Yst: yellow semi clear -Y: yellow- Yt: yellow turbid – Yst: yellow semi turbid- Yc: yellow clear).

## Discussion

In summary the results of urine analysis and urinary sediments depend on several factors likes being subjected to addiction abuse drugs, physiological states of a patients, age or consumption history and other lifestyles [13]. Additionally, there are vital and dead uroepithelial cells in fresh urine. However, the urine cells have been shown that urinary epithelial cells have vitality function in vitro cell cultures. Given these characteristic differences in the nature of uroepithelial cells in urine sediment and the fact that the majority of adult human diseases are derived from urine sediment, the study of urine samples have particular relevance toward understanding mechanisms of patients in humans. However, testing for presense of drugs in biological fluid samples also helps to improvement of patients in diagnosis and management. Because abuse drugs and their crystals are achievement in biological samples such as urine, therefore in this study we have not only just done to monitoring of urine macroscopic parameters by rapid dip stick tests but also confirmed of them in urine sediments. Light microscope application has been let's a broad spectrum of confirming for evaluation of urine analysis [13,14,15,20]. Urine analysis has been used for various diagnostics, including to pre-nal or urinary tract disease, kidney function, diabetes, pregnancy, and other hydration testing. A normal sample of urine consists of several chemicals' elements: urea, chloride, sodium, amino acids, sulphate, phosphate, potassium, and other trace number of various biomolecules. Not only abnormal levels of these substances with

atypical urine chemical properties and the presence of certain other chemicals or molecules can be used to diagnose of certain health conditions but also change in diseases. However, analysis of urinary specific gravity and pH can be used to monitor kidney functionality, renal tubular acidosis or alkalosis, hydration, and other urinary tract diseases. Furthermore, hematuria and proteinuria, for conditions such as Fabry's disease, nail patella syndrome, and several others are respectively indicators. In addition, certain proteins, urobilinogen, vitamins, ketones, nitrites, specific ions, bilirubin, or hormones in urine can be possible warning signs of specific disorders and screened as a biomarker. Physical properties that are commonly appeared include color, volume, specific gravity, and odor. Chemical properties that are often reported include pH, glucose, protein, ketones, bilirubin, urobilinogen, vitamins, blood, nitrites, and ascorbic acid. Furthermore, urine can be examined under the microscope to observe the presence of cells, crystals, bacteria, or other particles [7-13]. These dip stick strips are available for practical urine testing various substances, including specific gravity, acidity/alkalinity, protein, glucose, ketones, blood and blood components, including hemoglobin, white blood cells, nitrates, abuse drugs, and their metabolites [7]. Inorganic ions as sodium, potassium, calcium, magnesium, chloride, and phosphate are commonly found in urine. Abnormal levels of these ions can be key indicators in distinguish of diabetes, liver and kidney dysfunction, and acute or chronic cardiac failure, among other disorders. Specifically, abnormally high calcium levels may indicate endocrine disorders, cancers, or osteoporosis. Therefore, low calcium levels may be indicative of a vitamin D deficiency, parathyroid hormones dysfunction, or diseases relating to poor gut absorption of calcium [1,2,8-20].

## Conclusion

All Diagnostic devices, tests, urine laboratory practice or laboratory examinations and procedures that have to be approved by the FDA for home and out use. Because they are sufficiently simple and have insignificant risk [2]. However, it is clear that urine sediment examination provides a wealth of information about the patient's underlying renal disease. This study shows the importance of urine sediment examination in the diagnosis and management of kidney disease [1,8,9,12,13]. This study describes the importance of urine drug distinguish tests such as urine analysis by rapid tests. However, this epidemiological study supported by the clinical laboratory of welfare organization, hereby has been extremely helpful in quick assessment of drugs casts and drugs crystals in abusers' urines sediments [12,13,14,15].

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