

Evaluation of Common Adulterants in Morphine Urine Drug Screening

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Abstract

Background: Manipulating drug screens, changing sample quality, and confounding test results are common practices seen among opium users. These practices are intended to deceive drug screening systems using a wide variety of commonly available chemical compounds such as lemon juice and fertilizer. These actions create a challenge for addiction screening reference laboratories.

Objectives: Therefore, we studied the effects of lemon juice, nitrogen-based chemical fertilizers, carbon copy paper, and urine sample dilution on morphine urine screening test results.

Patients and Methods: We studied the urine samples of all individuals who were referred to the drug laboratory at Sabzevar public health center from August 2014 to August 2015. Out of 8012 urine samples tested in one year, morphine metabolites were identified in 493 (6%) of the urine samples using a rapid morphine slide test and thin-layer chromatography. Next, 180 confirmed positive samples were randomly assigned to three groups of 60 samples each, to which were added either carbon paper, lemon juice, or nitrogen-based fertilizer.

Results: The test results showed that in the carbon paper and lemon juice groups, 59 samples tested positive for morphine, and one sample was distorted. In the fertilizer group, 57 samples were positive and 3 were distorted. None of the groups showed negative test results. Moreover, negative results were not seen when testing standard samples that had been adulterated in the test kit's sensitivity range and higher. However, fertilizer and lemon juice adulteration, but not carbon paper, can lead to false negative results with lower morphine concentrations (150 ng/mL).

Conclusions: We found that adding nitrogen-based fertilizer, lemon juice, or carbon copy paper to urine samples does not facilitate false negative results when using a rapid slide morphine urine test. However, testing samples of various standard concentrations of morphine showed that at lower morphine concentrations, adding lemon juice or fertilizer can affect the test results.

Keywords: Morphine Test, Lemon Juice, Chemical Fertilizer, Confounding (Adulteration) Materials

1. Background

Most parts of the world are witnessing increased drug abuse, and drug abuse could be regarded as one of the foremost physical, psychological, and social challenges worldwide (1, 2). According to international organizations, about 4% of the world population is addicted to drugs, although informal statistics suggest different rates for global addiction. Addiction is a cultural and social taboo in Iran; therefore, accurate estimations of drug abuse rates are difficult. Positive urine test results from recruitment, marriage, and job application pretests in reference laboratories suggesting an increasing rate of drug abuse in Iran (1, 3, 4).

Narcotics have different effects on users, of which the most significant include analgesic and sedative effects as-

sociated with physical and psychological dependence. Precise laboratory tests are used to prevent family breakdown and assignment of addicts to civil services. Routine addiction tests are qualitative. Quantitative tests are also used to detect drugs and their metabolites (4, 5). Screening tests, such as staining, colorimetric tests, and thin-layer chromatography (TLC) are routinely used. Although confirmatory tests such as gas chromatography, high-performance liquid chromatography, immunoassays, and mass spectroscopy are more precise and sensitive, they require specialized and expensive devices (2, 3, 5-8).

Reference laboratories are facing the problem of false negative test results when screening for drugs. Drug users attempt different methods to hide their usage to create a false negative test result. Opium users use various

methods for this purpose including urine sample dilution, adding adulterants, and sample substitution (8-10).

Adulteration frequently refers to the addition of chemicals to a urine sample to change the test results (11). Some of the most common confounding materials are soap, lemon juice, household bleaching fluid, carbon copy paper, baking soda, oral contraceptives, and more recently, chemical fertilizer (7, 8, 12-15). Measuring the specific gravity of the sample with a refract meter can identify the addition of salt or sample dilution. Measurement of urinary creatinine levels can contribute to correct results. Some users believe that changing the urine pH can lead to negative urine screening test. They also believe that the blue color of carbon paper can stain the urine test's stick and therefore, produce a negative test result. Adulteration can violate the law and lead to the growth of addiction in the community by teaching the methods to other users, which undermines the efforts put in place for prevention of drug abuse, while increasing criminal acts in the country (7, 13, 15).

Every endeavor to better identify and discover solutions to prevent addiction is important. Because drug users employ various compounds to facilitate a negative drug test result, testing every single compound is not applicable and logical.

2. Objectives

Therefore, this study tests the effects of lemon juice, chemical fertilizer, and carbon paper on morphine test results to provide a better and easier method for identification of this cheating acts and their actors throughout the study' findings.

3. Patients and Methods

In this quasi-experimental study, we sought to identify the effects of lemon juice, chemical fertilizer, and carbon copy paper on morphine urine test results in the Sabzvar Medical University's public health laboratories. We studied the urine samples of all individuals who visited the Sabzevar drug reference laboratory in one year ($n = 8012$) from August 2014 to August 2015. The rapid slide drug test (Rapid Test Kit; CORE, China), with a diagnostic sensitivity of 300 ng/mL of morphine, showed positive results in 576 urine samples. Then, confirmatory tests were performed using TLC (Vira Nevin Teb, Iran), with a sensitivity of 1000 ng/mL of morphine. The presence of morphine metabolites were confirmed in 493 urine samples. The TLC solid phase was silicate, and the mobile phase was chloroform, ethanol and ammonia. Staining was performed using chloroplatinate. All positive samples were stored in

plastic containers in a freezer. At the time of the study analysis, 180 confirmed positive samples were randomly assigned to one of three groups, each containing 60 samples. These groups were designated as the carbon copy paper group, lemon juice group, and chemical fertilizer group.

All the urine samples were tested using a rapid stick test (Fastep, UK), with a diagnostic sensitivity of 300 ng/mL of morphine, and an immuno chromatography test to detect morphine.

Given the lack of a similar study to determine the amount of confounding material to add to urine samples, a group of drug users contributed in determining the amount and application of confounding materials with prior permission. One square centimeter of carbon copy paper (Pelican Co., Iran) was added to each sample in the first group, 0.5 mL of lemon juice was added to each sample in the second group, and 5 pearls (20 mg/mL) of chemical fertilizer was added to each sample in the third group. It should be noted that confounding materials were added based on the urine volume and impact on the urine color. On the day of the experiment, 5 mL from each sample was poured into a separate container after thawing the frozen urine, and tested. The presence of morphine metabolites was then confirmed for each urine sample, ruling out any effects of freezing and melting on the urine samples. Next, the specific gravity and pH of samples were measured using a refractometer and paper pH meter (Merck, Germany), and the above-mentioned amounts of confounding materials were added to the urine samples in each group. After addition of confounding materials, the specific gravity and pH of samples were measured again, and any differences were considered to be effects of the confounding materials. Then, morphine detection kits were applied to each urine sample. The standard morphine concentrations (Vira-Nevin Teb, Iran) were purchased and prepared in another laboratory of the basic science faculty of the Sabzevar medical university. To prepare the required concentrations, a pool of negative healthy urine samples was prepared and tested for the presence of morphine by rapid slide test kit and TLC. Then the lowest concentration of morphine that led to a stable positive result was determined through testing 50, 100, 150, 300, and 600 ng/mL concentrations four times using the rapid slide test kit. Again, the previously mentioned concentrations of confounding materials was added to the prepared morphine concentrations and tested. The rest was performed four times at each concentration. Considering the lack of information on the donors of individual samples, the donors' anonymity was preserved in a manner consistent with the university's code of research ethics.

4. Results

The screening tests were carried out on the 180 confirmed positive urine samples that had been randomly assigned to three groups of 60 samples each. The specific gravity and pH of samples was measured before and after adding adulterating material. Specific gravity showed a slight increase with the addition of chemical fertilizer, but not with carbon copy paper or lemon juice. Adding lemon juice acidified the samples (pH = 3), but there was no change in the samples' pH with the additions of carbon copy paper or fertilizer (Table 1).

The test results for the 60 samples in the lemon juice group showed that 59 samples remained positive and that one sample was confounded and remained so after repetition of the test. There was not any negative test result in this group. The confounded result meant that the stick did not change after being dipped in the urine sample and could be considered missing data. In such a case, the test should be repeated, but did not denote a negative result.

In the chemical fertilizer group, there were 57 positive results and 3 confounded results even after repeating the test. There were no negative test results.

In the carbon copy paper group, there were 59 positive results and 3 cases of confounded results after a repetition of the test. There were no negative test results.

After establishing different standard concentrations of morphine (150, 300, and 600 ng/mL) with a positive stable result, it was became clear that both kits (Vira-Nevin Teb, Iran and Fastep, UK) delivered similar results (Table 2). Carbon copy paper, lemon juice, and chemical fertilizer did not lead to negative results at the 300 or 600 ng/mL morphine concentrations. Addition of lemon juice and chemical fertilizer to 150 ng/mL of morphine concentration led to negative results in all four repeated tests. With the addition of carbon copy paper to the 150 ng/mL concentration sample, three out of four tests showed positive results, and one negative result was observed.

Chi-square tests were performed on the results of all three sample groups, to compare measures before and after the addition of adulterants. The chi-square test results showed that these confounding materials did not lead to significantly more negative morphine test results in any of the tests ($P = 0.212$).

In the case of standard samples, although these results were not observed in 300 and 600 ng/mL concentrations of morphine, but at lower than cutoff concentrations, lemon juice and chemical fertilizer led to a negative test result. Data were analyzed using the SPSS16 software.

5. Discussion

Sample manipulation or adulteration is one of the challenges that narcotic reference laboratories face. Unfortunately, in tandem with the development of new laboratory methods to detect drug abuse and addiction, counter-detection methods have been developed. Drug addicts uses different methods for changing positive results, including increasing usage of the confounding materials (8-10).

We studied the effects of lemon juice, chemical fertilizer, and carbon copy paper as confounding adulterants on urine drug screen results, and their effects on different standard concentrations of morphine in urine. The addition of lemon juice, chemical fertilizer, and carbon copy paper had no effects on the test results of positive urine samples. There were one and three confounding results in the lemon juice and chemical fertilizer groups, respectively. It seems that the reason for this difference could be the natural characteristics of the urine or kits and technician error; however, there was no possibility to resample or to contact the sampled person for a follow up test. Nevertheless, these methods did not lead to false negatives, even after repetition of the tests. Other studies have shown no effects for lemon juice, nitrite, and estrogen on morphine test results, either (7, 13, 16). Hedayati et al. studied different available household materials and reported that these material can lead to a false negative results due to changes in the urine pH and specific gravity (13). Addicts believed that swallowing carbon copy paper can accelerate the opioids metabolism or excretion and therefor, false negative results; but due to ethical limitation, tests were only conducted on the urine samples of the patients. Despite the strong beliefs that carbon copy paper would produce false negatives among drug users, our findings showed no effects for this method in terms of producing false negatives, which is consistent with the findings of other studies (7, 8).

It has been reported, that some drugs and foods could lead to errors in laboratory test results and lower their diagnostic sensitivity (5, 8, 17-22). Therefore, proper screening and confirmatory tests with high sensitivity should be performed for detection of the drug abuse (2, 5, 9, 17).

New guidelines from the reference laboratory of the Iranian Ministry of Health require the use of Iranian-made kits for drug screening tests. Comparing Iranian-made tests with different certified foreign test kits is therefore both necessary and useful. In this project, after confirming the positive samples, rapid stick test kits from both Iranian and foreign companies (Vira-Nevin Teb and Fastep) were used to assess adulterants in standard morphine concentration samples. The results showed that both kits could easily detect concentrations of morphine, even up to half

Table 1. Results of the Screening Tests Applied to Urine Samples, as Well as Changes in Specific Gravity and pH After the Addition of Confounding Materials

Confounding Material	The Urine Specific Gravity Change After the Addition of Confounding Material	The Urine PH Change After the Addition of Confounding Material	The Number of Positive Tests Before the Addition of Confounding Material	The Number of Positive Tests After the Addition of Confounding Material
Lemon juice	No change	Decrease	60	59
Carbon copy paper	No change	No change	60	59
Chemical fertilizer	Increase	Increase	60	57

Table 2. Screening Test Results Before and After Adding Confounding Materials to Different Standard Concentrations of Morphine

Concentrations	50		100		150		300		600	
	Before ^a	After ^b								
Lemon juice	0/4	-	2/4	-	4/4	4/0	4/4	4/4	4/4	4/4
Carbon copy paper	0/4	-	2/4	-	4/4	4/3	4/4	4/4	4/4	4/4
Chemical fertilizer	0/4	-	2/4	-	4/4	4/0	4/4	4/4	4/4	4/4

^aThe number of positive tests before the addition of confounding material.

^bThe number of positive tests after the addition of confounding material.

of the claimed cutoff level of the kits, namely 150 ng/mL. The performance of the Iranian-made test was comparable to previously-validated foreign-made kits in their consistency.

The relationships between the test kit’s sensitivity and confounding factors effects has not been explained in similar studies. Because of the lack of data on morphine levels in the samples used in the present study, different concentrations of standard morphine were prepared and tested. Results have shown that these confounding materials did not cause false negative test results at kit’s sensitivity level and higher, but at lower concentrations (150 ng/mL), chemical fertilizer and lemon juice led to false negative results. The test results for the kit’s sensitivity level were consistent with previous studies’ findings (11, 13, 14).

Finally, it can be argued that although the use of known confounding materials can be identified using parameters such as specific gravity, creatinine, and urine pH, but it is more logical to follow the cheating and adulteration detection approaches instead of detection of individual confounding materials. Therefore, due to ethical limitation of this project, it is suggested that these tests are carried out in vitro on a number of volunteer drug users with moral considerations; and in case of false negative results, the mechanisms shall be studied and any law escaping approach has been removed. Furthermore, studies on tests to detect other opioids and psychotropic substances (including methadone, amphetamine and methamphetamine) are necessary considering the usage and availability of these materials as well as the possibility of their inclusions in administrative inquiries, and should be considered in the future studies.

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Footnotes

Authors’ Contribution: Study concept and design, Sayedeh Masoumeh Hosseini Zare, Ahmad Ghalenovi, Maryamsadat Hosseini Zare and Mojtaba Fattahi Abdizadeh; acquisition of data, Sayedeh Masoumeh Hosseini Zare, Ahmad Ghalenovi, Maryamsadat Hosseini Zare; analysis and interpretation of data, Sayedeh Masoumeh Hosseini Zare, Mojtaba Fattahi Abdizadeh; drafting of the manuscript, Bahareh Vahidiyan Far, Mojtaba Fattahi Abdizadeh and Sayedeh Masoumeh Hosseini Zare; critical revision of the manuscript for important intellectual content, Mojtaba Fattahi Abdizadeh; statistical analysis, Mojtaba Fattahi Abdizadeh; administrative, technical, and material support, Bahareh Vahidiyan Far and Mojtaba Fattahi Abdizadeh; study supervision, Mojtaba Fattahi Abdizadeh.

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