Screening of Opioid Abuse in Toxicology Unit and Laboratory at Mansoura University with A Comparison of Two Detection Methods: Clinical and Legal Issues

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ABSTRACT

Background: Drug abuse is a critical concern worldwide. Urine drug screening is a common practice applied for the detection of common drugs of abuse for both clinical and legal issues. **Objective:** This study was performed to screen for opioid abuse in urine samples collected from adult subjects (aged > 18 years) at the toxicology laboratory and toxicology unit, Emergency Hospital, Mansoura University, and to compare enzyme-multiplied immunoassay (EMIT) with thin layer chromatography (TLC) techniques for confirmation of opiate in samples.

Patients and Methods: A total of 500 urine samples were randomly collected and screened with immunochromatographic (rapid) test strips for opiates. The positive urine samples obtained by the rapid test were further analyzed by EMIT and TLC techniques.

Results: A total of 80 samples (16%) were positive for opiate by the rapid test. By analyzing these positive samples using EMIT, only 68 samples were positive (68/80; 85%). Only 66 samples (66/80; 82.5%) revealed positive results (opiate spot) meaning that TLC was able to confirm 2 false-positive results by EMIT. This might be due to drug interactions.

Conclusion: Opioid abuse seems to be a problem among the Egyptian adult population. Despite the high sensitivity of EMIT (100%), TLC is more specific when compared with it. Indeed, EMIT can be a relatively accurate alternative for TLC, but, whenever positive results need to be re-checked, it is recommended to use the cheap and widely available TLC technique or ideally use the High-performance liquid chromatography (HPLC), particularly for legal purposes.

Keywords: Chromatography, EMIT, Opiates, TLC, Urine, Drug abuse.

INTRODUCTION

Substance abuse has become a serious public health concern, especially among the youth. In Egypt, drug abuse is considered one of the serious problems that worry both the people and government; however, epidemiological data on drug dependence are still few. Drug abuse and addiction are also serious problems that worry the Egyptian government, as it deals with young people within the age of work and productivity. It may lead to many problems such as bad social adaptation, decreasing productivity at work, or dismissal from the job [1].

Globally, including in Egypt, urine drug screening (UDS) is commonly required in pre-employment, suspicion of substance abuse in clinical settings, testing of employees, military services, sports participation, legal/criminal settings, marriage as well as postmortem investigation [2].

Urine drug screening has been the commonest method for analysis due to the ease of sampling and analysis. Such simplicity of usage and access to quick results has increased demand for and use of immunoassay techniques. In general, 5 different types of immunoassays are available: ELISA, EMIT, fluorescence polarization immunoassay (FPIA), immunochromatography, and radioimmunoassay (RIA). These assays are frequently used to detect amphetamines, cannabinoids, cocaine, opiates, phencyclidine, and benzodiazepines [3].

The immunoassay drug tests, which are designed to classify substances as either present or absent according to a predetermined cutoff threshold, are the most common methods. Immunoassays are based on the principle of competitive binding and use antibodies to detect the presence of a particular drug or metabolite in a urine sample [4].

Unfortunately, such immunoassays are not without disadvantages. False-positive results of immunoassays because of drug cross-reactivity can result in significant medical, legal and social concerns if results do not undergo confirmation by secondary more confirmatory methods, including TLC or HPLC [3].

Despite the unquestionable superiority of HPLC because of higher performance and automatization, TLC has many advantages such as simplicity, low cost and time for analysis, fewer solvents and reagents, and the capacity to simultaneously handle numerous samples. TLC is also attractive from "green chemistry" viewpoint. Lastly, TLC could be carried out without any complicated instrumentation. For these reasons, TLC is still extensively utilized and is one of the most common procedures for lipophilicity estimation, nowadays [5].

Therefore, the current work was performed to screen for opioid abuse in urine samples collected at the toxicology laboratory and toxicology unit, Emergency hospital, Mansoura University, Egypt, and also to

compare EMIT with TLC techniques for detection of opiates in urine samples.

MATERIAL AND METHODS
Material:
1. Rapid test for drug screening:
Immunochromatographic test strips were purchased for the preliminary urine screening (The cutoff was 300 ng/mL) (ABON Biopharm (Hangzhou) Co., Ltd., China).

Positive: The positive result indicates that the drug concentration in the urine sample exceeds the designated cut-off for opioids. This was indicated by an appearance of a colored line in the C region.

Negative: The appearance of a colored line in the C region and a colored line in the T region for a specific drug indicates a negative test result. The negative result indicates that the drug concentrations in the urine sample are below the designated cut-off levels for opioids.

2. TLC material:
Methyl alcohol, ammonia solution (25%), hydrochloric acid (25%), hexachloroplatonic acid, ethyl acetate, morphine hydrochloride reference standard, TLC plates (silica gel 20 cm×20 cm, 0.200 mm layer thickness), glass jar were used in this study.

3. Autoanalyzer for EMIT assay (Model: BioLis 24i, Tokyo Boeki, Japan).

Methods:
The collected urine samples were tested with immunochromatographic test strips (rapid test). The positive samples revealed by this rapid test (80 samples) were confirmed for opiate metabolites using EMIT and TLC techniques at the toxicology lab at forensic medicine and clinical toxicology department, faculty of medicine, Mansoura University. Analysis of the urine specimens was performed without delay (within 2 weeks).

Ethical consent:
This study obtained its approval from the institutional research board of Mansoura Faculty of Medicine (code: R.22.04.1693). Every patient signed an informed written consent for acceptance of participation in the study. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

1. EMIT:
For each urine sample, 5 ml of urine was poured into a plastic tube and was tested using an autoanalyzer. Before the operation, the autoanalyzer was calibrated according to the manufacturer's instructions using the specific reagents. Before any analysis, the autoanalyzer underwent conditioning for better performance. After that, the high and low opioid controls were analyzed before sample analysis to ensure that the autoanalyzer

was appropriately calibrated. The cutoff was 300 ng/mL.

2. TLC:
This was performed based on the methodology by **Ahadi et al.** [6] using liquid-liquid extraction. In brief, to 10 mL-aliquot of urine sample was added 0.5 mL of concentrated HCL, with subsequent heating for 15 minutes at 100°C to break glucuronide conjugates. The sample was then cooled and the pH underwent adjustment to 8-9 with ammonia. Extracting was performed with 5 mL of chloroform-isopropyl alcohol (4:1). The organic phase was separated and underwent evaporation to dryness.

The extract was dissolved in 100 μL of methyl alcohol and 5 μL of the obtained solution was spotted on a TLC plate using a micropipette. Furthermore, 5 μL of a solution that contained morphine standard was also spotted on the TLC plate to obtain a reference morphine spot to compare with the sample. These spotted plates were developed in a saturated TLC chamber of ethyl acetate: methanol: ammonia (85:10:5). TLC plates were then dried. The morphine-positive samples were recognized by visualizing morphine spots on the TLC plate after spraying via acidified iodo-platinate. Retention factor and the color of the spot (violet) were 2 main parameters to compare to that of morphine standard spot for determination of morphine-positive samples. Acidified iodo-platinate was prepared as described by **Wall** [7].

Statistical analysis
The collected data were coded, processed, and analyzed using the SPSS (Statistical Package for Social Sciences) version 23 for Windows® (IBM SPSS Inc, Chicago, IL, USA). Data were tested for normal distribution using the Shapiro Walk test. Qualitative data were represented as frequencies and relative percentages. Chi-square test ($\chi2$) to calculate the difference between two or more groups of qualitative variables. Quantitative data were expressed as mean ± SD (Standard deviation).

Independent samples t-test was used to compare two independent groups of normally distributed variables (parametric data). P-value < 0.05 was considered significant.

RESULTS
The current study included 80 positive samples (16%) for opiate metabolites using the rapid test for drug screening (**Table 1**). On analyzing these samples by autoanalyzer (EMIT technique), 68 samples out of the 80 samples were positive (86.25%). However, by using the TLC technique, only 66 samples (82.5%) were positive (colored spot).

A Positive result by TLC was revealed by the appearance of violet spot and by RF. The difference between TLC and EMIT was non-significant (P =0.66) (**Table 2**). In contrast, there was a high statistically

significant difference between either EMIT or TLC and rapid tests.

Table 1: Incidence of opioid abuse based on the rapid test.

No. of examined urine samples	No. of positive samples for opiate	No. of negative samples for opiate
500	80 (16%)	420 (84%)

Table 2: comparison between rapid tests, EMIT, and TLC.

Rapid test	EMIT		TLC		p-value
Positive	Positive	Negative	Positive	Negative	P1=0.0003*
80	68 (85%)	12 (15%)	66 (82.5%)	14 (17.8%)	P2<0.001* P3=0.66

P1: the difference between rapid test and EMT; P2: the difference between rapid test and TLC; P3: the difference between EMIT and TLC

Table (3) showed that EMIT had 100% sensitivity, 97.1% positive predictive value, and overall accuracy of 97.5 in comparison to TLC for detection of opiates in urine.

Table 3: Sensitivity, positive predictive value, and accuracy of EMIT in comparison to TLC.

Item	Percentage (%)
Sensitivity	100
Positive predictive value	97.1
Accuracy	97.5

DISCUSSION

Opioid drugs are commonly prescribed for pain and can also be used as illegal substances of abuse. Morphine and codeine are natural alkaloids obtained from opium poppy capsules [8].

This study showed that 80 urine samples out of 500 screened samples were positive for opioids by the rapid test with an incidence of 16% among those aged more than 18 years. This incidence is in agreement with **Eldabah et al.** [9] who reported that 17% of the examined urine samples were positive for opiates using the rapid test.

In addition and similar to our finding, **Goreishi and Shajari** [10] in Iran demonstrated that 16.5% of the examined students were abusers of codeine opioids.

Opiate immunoassay techniques classically target morphine and codeine. Semisynthetic opiates resemble morphine in structure, while synthetic opiates (e.g. tramadol) often necessitate separate immunoassays for screening [11].

In our work, the EMIT assay showed 100% sensitivity in detecting opiates in urine samples. However, only 2 urine samples were false positive by EMIT technique when compared with TLC (68 positive samples versus 66 positive samples, respectively). There was no statistically significant difference between both techniques in detecting urinary opiate metabolites (P=0.66). This demonstrates that EMIT possesses high sensitivity when compared with TLC. Conversely, our findings demonstrated a low specificity of EMIT in detecting morphine in urine samples.

TLC is a simple and one of the most versatile techniques for separating a mixture into its chemical components to isolate one compound or to evaluate the purity of the mixture. This is due to its reduced costs, being not time-consuming, highly sensitive, and good reproducibility [12].

According to manufacturers' claims, no cross-reactivity was found for many non-related medications to morphine derivatives at different concentrations in urine. Still, related compounds to morphine had been analyzed by the qualitative method and cross-reactivity could exist [13].

The false-positive results of EMIT might be because of drug interactions. In our study, the autoanalyzer was properly calibrated before analysis. Thus, technical failure (limitation of EMIT technique) can be ruled out.

According to our results, it is presumed that the EMIT assay has, although non-statistically significant, less specificity when compared with TLC, and thus it has the likelihood to give false-positive results. This was also demonstrated in other studies [14].

Some of these drugs (that give false-positive results) are utilized as prescriptions while some are deliberately utilized. Cross-reacting drugs may include naloxone and quinolones (Levofloxacine, ofloxacin, pefloxacin, lomefloxacin, moxifloxacin, ciprofloxacin, norfloxacin), verapamil, quetiapine, and diphenhydramine [15, 16].

The previously mentioned drugs could result in drug interaction mainly in the rapid strip tests and to less extent in EMIT leading to false positive or false negative results when compared with the TLC technique. This has coincided with **Timcheh-Hariri et al.** [13] who reported higher specificity of the TLC technique in detecting false-positive results by immunoassays.

Urine drug screens are commonly ordered by emergency physicians "to see if the patient is on something." A positive or negative urine drug screen is just a yes or no answer. The results of this test (positive or negative) should be interpreted with caution [17].

Finally, careful usage and accurate interpretation of toxicology testing in light of its limitations allow for judicious and therapeutic confrontation when illicit drug use is confirmed. Conversely, a lack of trust toward a patient can lead to inadequate/inappropriate treatments, erosion of the doctor-patient relationship, and even inaccurate diagnoses. The consequences of this lack of trust include patient behaviors that further jeopardize treatment and weaken the doctor-patient alliance while increasing monitoring and health care costs [15].

CONCLUSION

The current work highlights that opioid abuse seems to be a problem among the Egyptian adult population and also the significance of a cheap extensively available urine drug confirmation method like TLC to confirm any likely false-positive results obtained by immunoassays. Thus, whenever a positive result needs to be re-checked, it is recommended to use the cheap and widely available TLC technique or ideally by HPLC, particularly for clinical and legal purposes.

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