DIAGNOSTIC ACCURACY OF DRUG SCREENING IMMUNOASSAYS IN DRUG FACILITATED CRIMES

Sadia Dawood, Ayesha Hafeez*, Aamir Ijaz, Summera Moeen***, Asif Ali Memon****, Sumaira Mubarik*****

CMH Kharian Medical College, Kharian/National University of Medical Sciences (NUMS) Pakistan, *Combined Military Hospital Bahawalpur/National University of Medical Sciences (NUMS) Pakistan, **Rehman Medical Institute, Peshawar Pakistan, ***Shifa International Hospital, Islamabad Pakistan ****Armed Forces Institute of Pathology/National University of Medical Sciences (NUMS) Rawalpindi Pakistan, *****Wuhan University, Wuhan China

ABSTRACT

Objective: To determine the diagnostic accuracy of immunoassays in drug screening as required in emergency for the rapid diagnosis of drug intoxication in travel related crimes.
Study Design: Diagnostic accuracy study.
Place and Duration of Study: department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology, Rawalpindi Pakistan, from Jul 2017 to Jun 2018.
Methodology: Sealed urine specimens of 77 patients with history of suspected intoxication in drugs facilitated street crimes, received for toxicology screening were included in the study. All the specimens were analysed, initially on immunoassay (index test) and then on Triple Quadrupole Liquid chromatography–Mass spectrometry (reference standard). Benzodiazepine being the main class of drugs involved in travel related crimes, diagnostic accuracy of immunoassay technique was assessed for these by calculating its sensitivity, specificity, positive predictive value and negative predictive value.
Results: Victims were predominantly males and public transportation was the most common mode of transport. The most commonly used drug was Lorazepam. Immunoassay failed to detect few cases who were shown to be intoxicated with benzodiazepines by liquid-chromatography tandem mass spectrometry. The false negative rate was 4.9%. Only one false positive case was observed. The accuracy was calculated to be 94.8% with sensitivity of 95.08% and specificity of 93.7%.
Conclusion: Immunoassay was found reliable for rapid testing in drug facilitated intoxication cases. However critical decision making should be done cautiously keeping in mind the limitations associated with these screening procedures.

Keywords: Diagnostic accuracy, Drug facilitated crimes, Drug screening, Immunoassay.

INTRODUCTION

Use of drugs to incapacitate a person with the intention of criminal gain has occurred over the centuries, however reports of drug facilitated crimes have increased to greater extent during the past few decades[1]. Poisoning during travel has emerged as a new social and public health issue especially in the developing countries[2]. The challenge for physicians in treating such cases in the first place is to confirm the presence of any drug so as to be clear about the line of treatment. Urine drug testing (UDT) has shown to be a useful approach in identifying patterns of compliance, misuse, and abuse. Immunoassay (IA) based drug testing methods are widely used in clinical diagnostics due to their simplicity, low cost[3,4] and rapid detection time as compared to confirmatory techniques like Liquid-chromatography tandem mass spectrometry (LC-MS/MS). Sophisticated analytical techniques like LC-MS/MS involve long extraction process and complicated operation requiring technical expertise usually not feasible for emergency testing[5].

Significant controversy surrounds the diagnostic accuracy of UDT performed utilizing immunoassays vis-à-vis laboratory confirmation with LC-MS/MS. Previous researches have questioned the reliability of drug screening IA owing to increased chances of false positive and false

Correspondence: Dr Sadia Dawood, Department of Pathology, Armed Forces Institute of Pathology, Rawalpindi Pakistan
Received: 07 Aug 2019; revised received: 03 Jan 2020; accepted: 18 Feb 2020

negative results[6-9]. Different immunoassay techniques differ in their sensitivity and specificity which then also vary with the type of drug being analysed[10]. Knowing the specific shortcomings is essential for the accurate interpretation of the results of drug screening in emergency situations[11]. Moreover it may have a medicolegal implication as well. Manchikanti *et al* calculated the sensitivity and specificity of drug screening immunoassays for benzodiazepines analysis as 74.7% and 98.0% respectively[12].

With increase in incidence of travel related crimes arises the need for rapid recognition of symptoms and rapid detection/identification of the offending drug/class of drugs in order to exclude other conditions with similar symptoms and timely initiation of appropriate treatment. This testing assumes further significance in the setting of non-availability of clinical history. No local study is available that has assessed diagnostic accuracy of rapid drug screening immunoassays in such situations. So this study is planned to determine the diagnostic accuracy of immunoassays in drug screening as required in emergency for the rapid diagnosis of drug intoxication.

METHODOLOGY

This diagnostic accuracy study was carried out in the Department of Toxicology, Chemical Pathology, Armed Forces Institute of Pathology (AFIP) , Rawalpindi, from July 2017 to June 2018, utilizing non-probability, consecutive sampling.

Urine specimens of 77 suspected cases of drug intoxication in travel related crimes received at Toxicology department, Chemical Pathology, AFIP were recruited in the study, after approval from institutional review board (MP-CHP16-6/READ-IRB/17/391). We excluded cases (n=14) with incomplete clinical details, those for whom results of analytes BSR, ALT and Creatinine were not available, patients with un-conscious state secondary to causes other than drug intoxication and samples from known cases of chronic drug addiction. A well informed consent was obtained from the participants or their attendants in case of unconscious patients. History of the participants including, demographic data (age, gender etc), symptoms at presentation, mode of travel, mode of drug delivery was recorded in detail using a questionnaire. Results of the serum ALT, Creatinine and BSR were also obtained to exclude metabolic causes in an unconscious patient.

Sealed urine samples collected in sterile urine bottles were received with chain of custody form. All the urine specimens were also checked for the integrity of the specimen. Urine samples were analysed on Triage® TOX Drug Screen (Alere Diagnostics), a competitive fluorescence immunoassay designed for the qualitative determination of the presence of drug and/or the major metabolites above the threshold concentrations of up to 10 distinct drug classes, without any requirement of sample preparation. The QC device was run before running each batch.

Same samples were then hydrolysed and extracted prior to analysis on Triple quadrupole LC-MS/MS (QQQ/LCMS/MS). Analysis was performed on Agilent 6460 Triple Quadrupole LC-MS/MS (Agilent Technologies, USA), coupled to an electrospray ion source (ESI). The column used for the chromatographic separation was Poro shell 120 EC-C18 column (2.1 × 7.5mm. 7 micron, Agilent Technologies), heated at 55°C. Analysis was performed under multiple reaction mode (MRM), with a dwell of 50. Data acquisition and elaboration were performed by the Agilent Mass Hunter Workstation Software. A set of 9 calibrators plus 2 QC samples were run with each batch from 0.001mg/ml by diluting every standard with methanol. From the results of a pilot study conducted at our Institute it was found that benzodiazepine (BZD) was the main class of drugs involved in travel related DFCs. Previous researches were in concordance with this finding[13]. So current study focused on the diagnostic accuracy testing primarily for this major group of drugs. The prevalence of rest of the drugs in the immunoassay panel was too low to be the part of the present study. Cutoff in urine for BZD was 50 ng/ml. Calibrators were cerriliant certified and traceable to SI units. External quality control was

assured by Randox International Quality Assessment Scheme (RIQAS).

Analysis of data was done on statistical package for social sciences (SPSS 24). Diagnostic accuracy was assessed by calculating its sesitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and likelihood ratio. Qualitative data like age, mode of travel, and mode of drug delivery was analysed in terms of frequencies and percentages.

RESULTS

The study included 73 (95%) males and 4 (5%) females. Age of the study participants ranged from 16 to 65 years with mean age of 35 ± 12 years. Majority of the cases (90.9%) had been intoxicated while travelling on public transport as passengers, 7.8% were travelling on their private vehicles and 1.3% were pedestrian. All these cases had been offered food items usually drinks with the exception of two cases who couldn't recall any ingestion. A total of 57 (74%) patients were brought in an unconscious state whereas

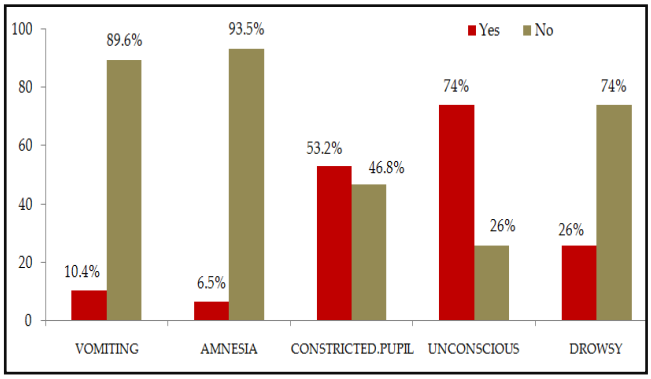

Figure-1: Percentage distribution of Signs/ Symptoms of patients on presentation.

remaining 20 (26%) were drowsy at the time of presentation. The signs and symptoms on presentation are described in the fig-1.

Results of serum ALT, creatinine and random blood glucose (BSR) were reviewed to rule out metabolic causes in unconscious patients. Mean serum creatinine, ALT and BSR were 87.3 ± 9.9 µmol/l, 32.8 ± 12.6 U/L and 4.9 ± 1.5 mmol/l respectively. Urine specimens from these 77 cases were tested by IA as well as on LC-MS/MS.

The false positive rate was 6.25% whereas the false negative rate was 4.9%. The IA gave results in the form of positive or negative for the entire class of benzodiazepines though each drug in the class react differently. Included in the three BZDs missed by IA were Lorezepam (n=1), a

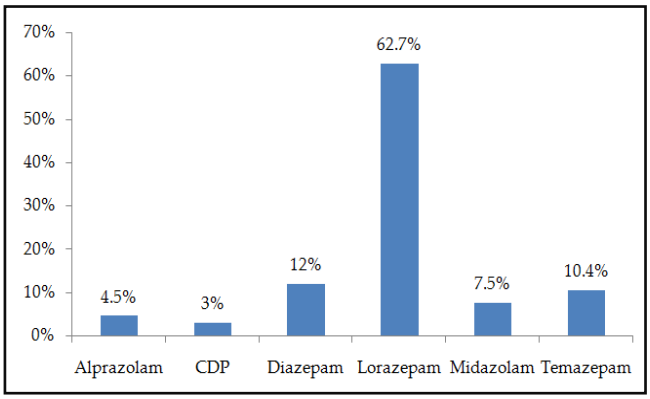

Figure-2: Percentages of types of benzodiazepines identified.

com-bination of Lorazepam and CDP (n=1) and a combination of Midazolam, Diazepam and CDP (n=1). In the specimen containing Lorazepam, drug concentration was lower than the IA cutoff. In remaining two of these three false negative cases, drug concentration calculated by mass spectrometry was much above the IA cutoff. So these false negative observations were due to poor cross reactivity of the IA and not due to the difference between the IA and LC-MS/MS cutoffs. Types of BZD identified on LCMS/MS in the positive cases are shown in fig-2. Diagnostic accuracy testing of IA in DFCs is shown in a flow diagram in table-I.

Overall diagnostic accuracy of BZD screening IA was assessed by the parameters given in

Table-I: Flow diagram of accuracy of Benzodiazepines testing

Statistic	Value
Sensitivity	95.08%
Specificity	93.7%
Positive Likelihood Ratio	15.21
Negative Likelihood Ratio	0.05
Positive predictive Value	98.31%
Negative Predictive Value	83.33%
Accuracy	94.81%

the table-II. There was no statistically significant association between the false IA results and the patient's demographic characteristics.

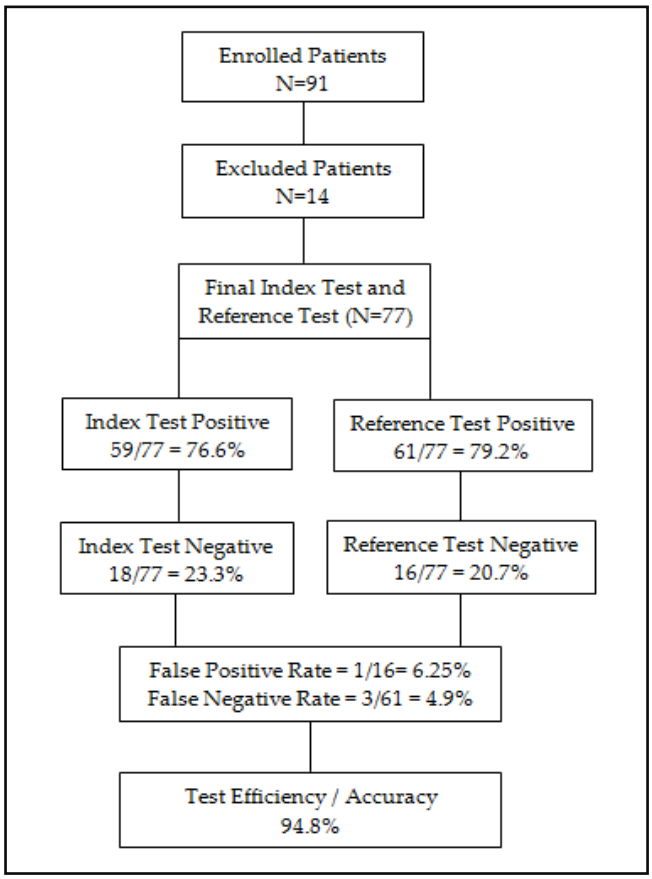

Table-II: Diagnostic accuracy of drug screening immunoassays for benzodiazepenes.

DISCUSSION

Rapid drug screen serves an important role in the diagnosis and management of critically ill poisoned patients. However clinical utility can only be attained if it is done as a part of stat/ urgent testing. Cases from drug facilitated travel related poisoning comprises major bulk of the emergency toxicology testing performed in our laboratory. We aimed to test the accuracy of these rapid drug screening immunoassays in emergency situations as no local study was available that could assess the accuracy of rapid testing screens used for such cases.

Two significant limitations of BZDs IA described in medical literature are poor cross reactivity with the conjugated metabolites and the higher cutoffs[14-16]. In the present study we observed three false negative results. In one of these Lorazepam was detected where the concentration of the drug was much lower than the cutoff. However it's not appropriate to comment on finding very low concentration in the scenario of heavy intoxication as urine is not suitable type of specimen to provide correct estimation of the amount of drug taken. In rest two cases negative results were attributed to poor cross reactivity with the drug. One of these two specimens contained a combination of Lorazepam and CDP and the other contained combination of Midazolam, Diazepam and CDP. It was found that CDP was never detected by immunoassay even when its concentration was above 600 ng/ml. The minimum threshold concentration of CDP required to give positive result is very high, i.e. 13,000 ng/ml (as mentioned by the manufacturer in the package inserts). This poor sensitivity for CDP was responsible for some of the false negative results. In one of the three false negative cases, concentration of Lorazepam was much higher above the cutoff. It showed that Lorazepam was sometimes not detected by IA. Poor cross reactivity of lorazepam has previously been described in many studies. Amadeo Pesce described 22% false negative rate for benzodiazepines; all of which were due to poor cross reactivity with Lorazepam[8]. Most IA could not detect the newer drugs (e.g Lorazepam, Alprazolam etc) which are excreted as their glucoronide conjugates. However this was contrary to our findings where most of the cases (91%) of Lorazepam were detected by IA. Another limitation of POC devices is that the cross reactivity data provided by the manufacturer does not include that of excreted metabolite of all the drugs[17]. Manufacturer insert of Alere Triage® TOX Drug Screen, utilized as IA screen in current study provides published data regarding the cross reactivity of Alprazolam glucoronide, Lorazepam glucoronide, Temazepam glucoronide and Oxazepam glucoronide etc, however the complete data regarding cross reactivity with the metabolite of all the drugs is lacking.

Sensitivities of immunoassays vary in various immunoassay techniques. Bertol *et al* when compared the main immunoassay methods used for forensic purposes with LC-MS/MS, observed highest number of false negative for FPIA where as the rest of the techniques provided good sensitivity and accuracy[18].

Kurisaki *et al* evaluated the Triage benzodiazepine assay and reported that it has low sensitivity in detecting Estazolam, Brotizolam, and Clotiazepam[19].

Current study demonstrated one false positive observation. The findings were similar to Machintaki who described the false positive rate of 2% for BZD. He was of the opinion that though confirmational may be required in few cases, immunoassay still provides an effective, reliable and rapid means for drug screening[12]. Previous researches have revealed the role of potential interferents including certain drugs such as Efavirenz, Sertraline, Oxaprozin and Tolmetin in causing false positive interference with BZD assay[20-23]. We obtained 16 negative results on LC-MS/ MS. One of these patients presented late for the toxicology screening and probably time had passed beyond the detection window for the drug. Another patient among the negative cases was negative probably because not enough time had passed for the drug to appear in urine as his gastric fluid, though not the part of the study was positive for benzodiazepines.

One interesting finding of the study was that Lorazepam was the most frequently used drug. Similarly in a study conducted by Basher A and his colleagues, Lorazepam was found in all the 22 specimen analysed[13]. Lorazepam is considered as a weapon of offence. Any one in the illegal possession of Lorazepam faces penalty under Massachusetts law. However in a country like ours, these drugs are easily available over the counter, for medical purposes. Diazepam, Temazepam, Midazolam, CDP and Alprazolam were the other types of BZD identified in few cases. However the type of BZD ingested has no effect on the management which remains the same in every case.

Even though the present study demonstrated that IAs have fairly good sensitivity and specificity but there were few false negative observations which can have serious consequences. These false observations can be attributed to higher cutoffs of IA, poor cross reactivity or patient metabolizing the drug in an unusual way etc. Manufacturers should overcome these limitations by lowering the cutoffs and addressing the problems of poor cross reactivities and the problems of false positivity by adapting newer methods in order to increase the efficiency of these drug screening platforms.

Clinician may not be aware of the limitations of IA and the variations in the sensitivities of various types of IA used by the laboratories. In the present scenario the negative result may be interpreted as the absence of drug which again can have grave consequences. We believe that individual laboratories should also guide the clinician about the limitation of these tests. According to the National Academy of Clinical Biochemistry while using IA if a positive result is reported, the laboratory should list the major cross reacting substances and in negative report it should be mentioned in the notes that negative result does not mean absence of all drugs of abuse[24].

The study was limited by the fact that only one IA diagnostic product was used and single laboratory was used. The results show the validity of drug testing in emergency settings in a particular setup and are not generalized to all health care settings.

Disclaimer

This article is a part of thesis project of Mphil in Chemical Pathology. This manuscript has not been published elsewhere and is not under consideration by another journal.

Funding Disclosure

The authors wish to thank Pakistan Health Research Council for funding the project.

CONCLUSION

Urine drug screens utilizing IAs are rapid, convenient and effective means of evaluating drug intoxication in emergency situations. However there are certain shortcomings associated with the interpretation of urine drug screens. It is important for the health care professionals to have knowledge of all the factors that influence the test results and the limitations of these screening platforms. When used in adjunct with the clinical assessment, they provide valid and reliable means for critical decision making.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

REFERENCES

1. Kumar A, Verma A, Jaiswal K, Kumar S. Emergence of entirely new poisoning in rural India; An upcoming health hazard to the community health. Indian J Comm Health 2012; 24(3): 248-51.
2. Khan TM, Mehr MT, Ullah H, Abrar A. Drugs-facilitated street and travel related crimes: a new public health issue. Gomal J Med Sci 2014; 12(4): 1-5.
3. Hage DS. Immunoassays. Anal Chem 1999; 71(12): 294-304.
4. Vanderlaan M, Stanker LH, Watkins BE, Petrovic P, Gorbach S. Improvement and application of an immunoassay for screening environmental samples for dioxin contamination. Environ Toxicol Chem 1988; 7(11): 859-70.
5. Gjerde H, Øiestad EL, Christophersen AS. Using biological samples in epidemiological research on drugs of abuse. Norsk Epidemiologi 2011; 21(1): 55-59.
6. Standridge JB, Adams SM, Zotos AP. Urine drug screening: a valuable office procedure. Am Fam Physician 2010; 81(5): 635-40.
7. McCarberg BH, Kirsh KL, Passik SD. Clinicians' perspective on the use of immunoassay versus definitive laboratory quantitation methodologies for medication monitoring. J Pain Palliat Care Pharmacother 2014; 28(3): 255-58.
8. Methadone MM, Codeine H, Hydromorphone M. An evaluation of the diagnostic accuracy of liquid chromatography-tandem mass spectrometry versus immunoassay drug testing in pain patients. Pain Physician 2010; 13: 273-81.
9. McDonell MG, Angelo F, Sugar A, Rainey C, Srebnik D, Roll J, et al. A pilot study of the accuracy of onsite immunoassay urinalysis of illicit drug use in seriously mentally ill outpatients. Am J Drug Alcohol Abuse 2011; 37(2): 137-40.
10. Vincent EC, Zebelman A, Goodwin C. What common substances can cause false positives on urine screens for drugs of abuse? Clinical Inquiries, 2006 (MU). 2006.
11. Moeller KE, Kissack JC, Atayee RS, Lee KC. Clinical interpretation of urine drug tests: what clinicians need to know about urine drug screens. Mayo Clin Proc 2017; 92(5): 774-96.
12. Manchikanti L, Malla Y, Wargo BW, Fellows B. Comparative evaluation of the accuracy of benzodiazepine testing in chronic pain patients utilizing immunoassay with liquid chromatography tandem mass spectrometry (LC/MS/MS) of urine drug testing. Pain Physician 2011; 14(3): 259-70.
13. Basher A, Faiz MA, Arif SM, Khandaker MAK, Kuch U, Toennes SW. Toxicological screening of drug facilitated crime among travelers in Dhaka, Bangladesh. Asia Pac J Med Toxicol 2017; 6(2): 47-49.
14. Moeller KE, Lee KC, Kissack JC. Urine drug screening: practical guide for clinicians. Mayo Clin Proc 2008; 83(1): 66-76.
15. Melanson S. KIMS, CEDIA, and HS-CEDIA immunoassays are inadequately sensitive for detection of benzodiazepines in urine from patients treated for chronic pain. Pain Physician 2014; 17(1): 359-66.
16. Mullins GR, Reeves A, Yu M, Goldberger BA, Bazydlo LA. Improved clinical sensitivity of a reflexive algorithm to minimize false-negative test results by a urine benzodiazepine immunoassay screen. J App Lab Med 2018; 2(4): 555-63.
17. Mikel C, Pesce AJ, Rosenthal M, West C. Therapeutic monitoring of benzodiazepines in the management of pain: current limitations of point of care immunoassays suggest testing by mass spectrometry to assure accuracy and improve patient safety. Clinica Chimica Acta 2012; 413(15-16): 1199-202.
18. Bertol E, Vaiano F, Borsotti M, Quercioli M, Mari F. Comparison of immunoassay screening tests and LC–MS-MS for urine detection of benzodiazepines and their metabolites: Results of a national proficiency test. J Anal Toxicol 2013; 37(9): 659-64.
19. Kurisaki E, Hayashida M, Nihira M, Ohno Y, Mashiko H, Okano T, et al. Diagnostic performance of Triage™ for benzodiazepines: urine analysis of the dose of therapeutic cases. J Anal Toxicol 2005; 29(6): 539-43.
20. Nasky KM, Cowan GL, Knittel DR. False-positive urine screening for benzodiazepines: an association with sertraline?: a two-year retrospective chart analysis. Psychiatry (Edgmont) 2009; 6(7): 36-39.
21. Blank A, Hellstern V, Schuster D, Hartmann M, Matthée AK, Burhenne J, et al. Efavirenz treatment and false-positive results in benzodiazepine screening tests. Clin Infect Dis 2009; 48(12): 1787-89.
22. Camara PD, Audette L, Velletri K, Breitenbecher P, Rosner M, Griffiths WC. False-positive immunoassay results for urine benzodiazepine in patients receiving oxaprozin (Daypro). Clin Chem 1995; 41(1): 115-16.
23. Saitman A, Park HD, Fitzgerald RL. False-positive interferences of common urine drug screen immunoassays: a review. J Anal Toxicol 2014; 38(7): 387-96.
24. Wu AH, McKay C, Broussard LA, Hoffman RS, Kwong TC, Moyer TP, et al. National academy of clinical biochemistry laboratory medicine practice guidelines: recommendations for the use of laboratory tests to support poisoned patients who present to the emergency department. Clin Chem 2003; 49(3): 357-79.