

The frequency of polydrug use in a driving population in Rome

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ABSTRACT

In Italy the illicit substances routinely tested are cannabinoids, cocaine, opiates, amphetamines / methamphetamine, MDMA and similar but these substances are not the most use in our country. In particular, the consumption of ketamine represents an emerging problem. Ketamine is an anesthetic with hallucinogenic and dissociative effects and these are the ones sought for the voluptuary purpose, while the amnesic effect are exploited for drug facilitated sexual assault. Our study was carried out to assess the positivity for the illicit substances routinely tested and also for ketamine in a population of 182 patients arrived at the emergency room of the Hospital "San Camillo Forlanini" of Rome, for which have been required toxicological tests on the basis of Articles 186 and 187 of the New Highway Code. The choice of this kind of population allows to have an accurate and reliable epidemiological data about the real diffusion of voluptuary use of drugs.

The study examines 182 samples subjected to routine toxicological investigations in the period between October 2011 and August 2012. The Authors have researched the presence of ethanol, cannabinoids, cocaine, opiates, amphetamines and benzodiazepines with the use of "ADVIA Chemistry Systems", while the ketamine is tested by a single-phase tests on urine [Sure Screen Diagnostic (Di. Ra.Lab)] with a cut-off level of this method is 1000ng/ml.

During this period we have considered 182 patients (males 78%, females 22%). The mean age was 34 years (standard deviation 13, minimum 15, maximum 80). Most of the admission were during the last days of the week (Thursday 17%, Friday 15%, Saturday 15% and Sunday 17%). With respect to the clinical needs of subjects admitted, 42% were admitted with a red code.

Considering a single substance analysis, 46 subjects resulted positive to alcohol (25%), 38 to cannabinoids (21%), 24 to opioids (13%), 20 to cocaine (11%), 19 to benzodiazepine (10%) and 7 to ketamine (4%).

Among those who resulted positive to at least one substance (95, 52% of the sample) 55 subjects resulted positive to one substance (58%), 27 to two substances (28%) and 13 to three or more substances (13%). Poly-use was evaluated with an indicator previously proposed by Fabi et al in 2013. This resulted in a polydrug-use score equal to 2.93 (standard deviation 1.7, minimum 1.5, maximum 9.25).

Key words: polyuse, emergency rooms, driving population

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INTRODUCTION

Driving under the influence of alcohol, or other narcotic or psychotropic substances is currently an increasing problem. The countries of the European Union have not yet agreed upon a common legislative policy regarding driving under the influence of alcohol, or other narcotic or psychotropic substances, and as a result each country legislates for this independently, regulating and penalising differently. In Italy, new traffic laws have established under articles 186 and 186 (2) that a driving under the influence of alcohol is punishable by law; similarly, under article 187 a driver found to be in an altered state of consciousness due to taking either narcotic or psychotropic substances may also be punished by the law.

Ascertaining whether or not a driver is under the influence of drugs or alcohol may initially be done using qualitative non-invasive methods at the roadside, and following that through taking samples of body fluids in hospital. In Italy, as a matter of routine, samples are screened for cannabinoid, cocaine and opiate metabolites, as well as amphetamine/metamphetamine, MDMA and the like; however, these are not the only substances used in Italy today. Indeed, according to Italian epidemiological data, the use of ketamine, in particular, is an emerging problem.

Ketamine is an arylcycloalkylamine, that is an amine bound to a benzene structure and a non-benzene structure defined as alicyclic. Ketamine is a synthetic molecule, it was created in the laboratory in 1962, and patented in Belgium in 1963 for clinical use as an anaesthetic and analgesic. In fact, in the 1960s a new branch of anaesthesia called *dissociative anaesthesia* was formed due to the ability to cause an 'out of body experience', inducing a loss of response to pain stimuli, as well as to the surrounding environment. First, phencyclidine and then ketamine was used with exactly this aim. The state of anaesthesia produced by ketamine was first described in terms of a functional and electrophysiological disassociation between the thalamo-neocortical system and the limbic system. Ketamine's primary effect is upon the brain's thalamo neo-cortical projection, where the neurons of the cerebral cortex and the thalamus

are selectively inhibited, and at the same time, those of the limbic system, including the hippocampus are stimulated. This creates a situation of 'functional disorganisation' of the brain, which induces anaesthesia. The mechanisms by which this substance interacts with the organism are varied. The most relevant is the alteration of the glutaminergic system. Ketamine principally acts as a non-competitive antagonist to the NMDA receptor, binding to the binding site for inhibitors or antagonists, which is the same one to which PCP binds. From a functional point of view, the NMDA receptors are characterised by a slow open time and inactivation which entails the production of a flow of calcium ions responsible for a cascade of transduction reactions of the signal essential for the processes of synaptic plasticity, and also in the event of excessive calcium ions entering the cell, excitotoxic neural death. By binding itself to the antagonist sites, ketamine prevents the channel from opening, and therefore also the passage of Ca^{++} ions. Consequently, depolarisation does not occur, and the transmission of the signal from the outside to the inside of the cell is blocked. Thus, the drug acts as an analgesic, reducing or eliminating completely the sensation of pain. As with every other type of narcotic substance, its effect in qualitative terms and its duration of action is strictly determined by the quantity taken and the method of ingestion. Ketamine is capable of generating various different states of altered consciousness, and it is exactly for this reason that it is used in the quest for new sensory experiences and recreationally. Different doses and types of administration are used depending on the effect that the consumer would like to obtain. The most common ingestional methods used recreationally are oral or nasal ingestion, because they allow the psychedelic effects to appear rapidly, even if their duration is reduced. In fact, for recreational use, only medium to low doses of the drugs are used, because the physical incapacity that is produced as a result of large doses of the drug renders the consumer unable to interact with the surrounding environment, thus nullifying the recreational experience. In order to explore new sensory experiences, the drug is taken with the aim of inducing psychedelic effects; consequently, the dose is very important as it must allow for the consumer to remember the experience. On the

other hand, the anaesthetic and amnesic effects of this substance is often used with the aim of committing sexual violence. The pshychedelic quality of ketamine is very different to that of other hullucinogens like LSD, mescaline and psilocybin. While traditional psychedelic drugs tend to distort reality, generting unreal images, with ketamine the brain is deprived of all sensory information and points of reference in the external world, in this way realty disapperas, and a parrellel reality is created. An important property of this drug, is its capacty to produce, in some consumers, if taken at high doses, a *near death* experience. This type of experience is defined in slang as "K hole" because the consumers describe feeling "being sucked into a black hole at the speed of light". The unusual characteristics and properties of this substance have provided for an increase in those people who take it recreationally and who could, therefore, find themselves at the wheel of a car under its effects.

The aim of our study is to invesitage levels of positivity for routinely screened for narcotic substances and ketamine, in a population of subjects for which a chain of custody had been started with regard to articles 186, 186 (2) and 187 of the new traffic laws, and for which the terms of custody for the sample had expired (12 months). The chains of custody of interest for this study refer to requests of toxicological examination for medicolegal purposes for 182 patients at the Accident and Emergency Department of A.O. San Camilli-Forlanini between October 2011 and August 2012. This population was chosen in order to obtain more pertinent epidemiological data regarding the real extent of the abuse of narcotics, since the driver was neither aware of the possible checks on behalf of the authorities nor involved in a traffic accident.

MATERIALS AND METHODS

The samples under chain of custody procedures at the U.O Laboratorio Analisi di Biochimica Clinica dell'A.O. San Camillo-Forlanini are routinely analysed using Siemens ADVIA Chemistry Systems. Screening for cocaine, cannabinoid, amphetamine, opiate and benzodiazepine metabolites in the urine is performed using a competitive homogenous enzyme immunoassay. This method is based

on the competition between the substance present in the sample and that labelled with Glucose-6-phosphate dehydrogenase (G-6-PD) for a fixed number of binding sites for the specific antibody. If the substance is not present in the sample, the specific antibody binds to G-6-PD inhibiting enzymatic activity. An increase in concentration of the substance of abuse in the sample, which competes with that bound to the enzyme for the active sites on the antibody, results in an increase in enzymatic activity. The active enzyme converts nicotinamide adenine dinucleotide (NAD) into NADH dehydrogenase, causing an increase in absorption which is measured spectrophotometrically at 340/410 nm. The concentration of the substance is proportional to the increase in absorption. Screening for ethanol in blood samples, however, is achieved using an enzymatic method in which alcohol dyhydrogenase catalyses the conversion of ethanol and NAD⁺ analogue into acetaldehyde and NADH analogue. The high oxidizing potential of the NAD⁺ analogue keeps the reaction going. The speed of the increase in absorption at 350/505 nm is due to the reduction of the NAD⁺ analogue in the NADH analogue, and is proportional to the amount of ethanol in the sample. Since ketamine does not react with common reagents like Marquis, screening for it is performed using a single urine test [Sure Screen Diagnostic (Di.Ra.Lab)] based on a competitive immunoassay. This test uses particles of monoclonal rat antibodies which correspond to the protein conjugates of ketamine with the aim of showing 2 – 4% of the drug excreted in an unaltered form with a cut off of 1000ng/ml being sufficient to reveal it. The ketamine present in the sample competes with its relative conjugate for the same binding sites on the antibody. Urine moves along the membrane by way of capillarity, and if the substance is present in concentrations less than the cut off, it will not be able to saturate all the binding sites of its specific antibodies which react, therefore, with the conjugate protein. The formation of a coloured line visible in the reactive area indicates the absence of competition due to a concentration of ketamine in the sample lower than the cut off. If the drug is present in concentrations that are higher than the cut off, then all the binding sites of the antibody are saturated and consequently, no coloured line may be seen

due to competition between the two substances for the same antibody. To check that the test has been successful, a band will appear in the control zone containing goat antibodies which indicates that the correct amount of sample has been used and that the sample has moved along the membrane.

Also, considering that 6 different substances were screened at the same time, in subjects that resulted positive to the use of more than one substance, poly-drug use was evaluated. Poly-drug use was defined as concurrent use of more than one substance in a specific time period. Considering that the detection times depend mainly on the dose and sensitivity of the method used and also on the preparation and route of administration, the duration of use (acute or chronic), the matrix that is analyzed, the molecule or metabolite that is looked for, the pH and concentration of the matrix (urine, oral fluid), and the interindividual variation in metabolic and renal clearance. In general, the detection time is longest in hair, followed by urine, sweat, oral fluid, and blood. In blood or plasma, most drugs of abuse can be detected at the low nanogram per milliliter level for 1 or 2 days. In urine the detection time of a single dose is 1.5 to 4 days. In Particular the detection time of amphetamine in urine is generally accepted to be 1 to 3 days instead the main metabolites of cocaine is positive in urine for 1 to 2 days after intravenous administration of 20 mg after a higher dose (1.5mg/kg) taken intranasally, the detection time is 2 to 3 days. For Opiates the Maximal detection time in urine in 11.3 days. The detection time of cannabinoid in urine after a subject smoked a joint containing 1.75% THC, is 2.1 days instead after a joint with 3.5% THC the detection time is 3.8 days. In chronic users of cannabinoid, the inactive metabolite (THCCOOH) can be detected for weeks or even months. The longest reported detection times are 93 days and 95 days, but these are probably exceptional cases. Determination of alcohol is probably the most frequently and requested test performed by forensic laboratories. Many studies have shown that "alcohol has been found to be the largest single factor leading to fatal crashes". Any Blood Alcohol Concentration (BAC) measurements, therefore reflects not only a person's drinking rate but also his or her rate of metabolism;

Factor that influenced BAC during and after drinking a given amount alcohol include age, gender, the proportion of body mass made up by fatty tissue, and whether food is eaten with the alcoholic drinks. Ketamine is not a routine drug in urine immunoassay screen for drugs of abuse. The detection window for ketamina and norketamina in urine is dependent both on the analytic method and individual toxicokinetics. The detection time of ketamine urine concentration is estimated at 2-4 days.

The poly-drug use was evaluated by a score (poly-drug score or PDS) introduced in. Briefly, PDS for an individual was computed by summing for each substance used its harm weight, denoted by W. As previously discussed, W can be considered as a measure of global harm that considers different aspects of negative health consequences of a substance use: acute toxicity (X), chronic toxicity (Y) and dependence (Z). For the substances here considered we had: alcohol 2.18, benzodiazepine 1.31, cannabis 1.18, cocaine 2.07, ketamine 1.05, opioids 2.51.

Statistical analysis was performed using R 15.0 software.

RESULTS

During the period considered (Table 1) 182 person (males 78%, females 22%) were admitted to the Emergency Department of San Camillo Hospital in Rome. The mean age was 34 years (± 13). Most of the admission were during the last days of the week (Thursday 17%, Friday 15%, Saturday 15% and Sunday 17%). With respect to the clinical needs of subjects admitted, 42% were admitted with a red code.

Considering a single substance analysis, 46 subjects resulted positive to alcohol (25%), 38 to cannabinoids (21%), 24 to opioids (13%), 20 to cocaine (11%), 19 to benzodiazepine (10%) and 7 to ketamine (4%).

Among those who resulted positive to at least one substance (95, 52% of the sample) 55 subjects resulted positive to one substance (58%), 27 to two substances (28%) and 13 to three or more substances (14%).

Mean poly-drug use score was equal to 2.93 (± 1.7); considering the days of the week, the higher mean PDS value were on Friday (3.4 ± 2.2), on Sunday (3.4 ± 1.74), on Thursday (3.1 ± 2.2)

TABLE 1

| DESCRIPTIVE STATISTICS FOR THE SAMPLE ANALYSED. RESULTS ARE PRESENTED AS FREQUENCY (PERCENTAGE) | |
|-------------------------------------------------------------------------------------------------|---------------|
| | FREQUENCY (%) |
| GENDER* | |
| F | 39 (22) |
| M | 140 (78) |
| DAYS OF THE WEEK | |
| Monday | 27 (15) |
| Tuesday | 19 (10) |
| Wednesday | 21 (12) |
| Thursday | 31 (17) |
| Friday | 27 (15) |
| Saturday | 27 (15) |
| Sunday | 30 (16) |
| SUBSTANCES | |
| Alcohol | 46 (25) |
| Benzodiazepine | 19 (10) |
| Cannabinoids | 38 (21) |
| Cocaine | 20 (11) |
| Ketamine | 7 (4) |
| Opioids | 24 (13) |
| None | 87 (48) |
| POLY-DRUG USE | |
| 1 substance | 55 (58) |
| 2 substances | 27 (28) |
| 3 or more than 3 substances | 13 (14) |
| ADMISSION CODE | |
| Red | 77 (42) |
| Green | 68 (37) |
| Yellow | 32 (18) |
| White/other | 5 (3) |

*3 missing values in gender information

TABLE 2

| DESCRIPTIVE STATISTICS FOR AGE AND POLY-DRUG USE SCORE | | | | |
|--------------------------------------------------------|------|----------|------|------|
| | MEAN | ST. DEV. | MIN | MAX |
| AGE | 34 | 13 | 15 | 80 |
| POLY-DRUG USE SCORE (PDS) | 2.93 | 1.7 | 1.05 | 9.25 |
| POLY-DRUG USE SCORE (PDS) NO ALCOHOL | 2.52 | 1.7 | 1.05 | 7.07 |

and on Saturday (2.9 ± 1.5). According to the admission code, those with red code had the higher PDS (3.4 ± 1.9). By gender, PDS was similar (2.9 ± 1.9 for women, versus 3 ± 1.6 for men).

DISCUSSION

The heterogeneity age of the population, selected on the basis of the possible control of law enforcement, raises the average age (34) and tends to underestimate the actual incidence of the use of ketamine restricted to one age group more young. The figure of the actual use of ketamine is further underestimated because the data studied are related to emergency general and do not take into account the possible use of ketamine for sexual violence because these patients belong to the emergency room obstetrico-gynecological. The results show that cannabis has a high prevalence in this sample as cocaine and opioids among illegal substances and poly-use is rather frequent. It must also taken into account the presence of ketamine. This confirms other studies and, in particular the prevalence of ketamine as shown in various dealing data. It can be seen in Figure 1 (a, b, c, d) that it is more usual for dealers ketamine offering.

FIGURE 1A

PERCENTAGE OF THE CANNABIS USERS WHO DECLARE THAT THEIR DEALERS SELL ALSO OTHER SUBSTANCES

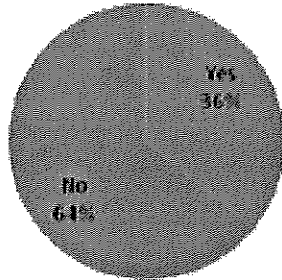


FIGURE 1B

FREQUENCY OF DEALING THE OTHER SUBSTANCES TOGETHER WITH CANNABIS

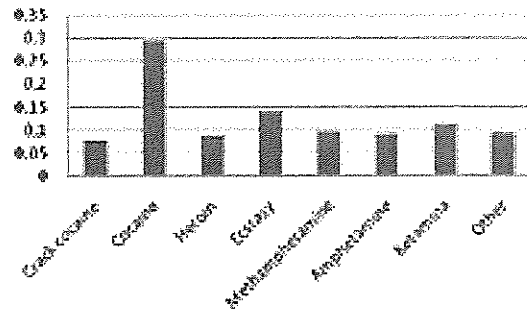


FIGURE 1C

PERCENTAGE OF THE COCAINE USERS WHO DECLARE THAT THEIR DEALERS SELL ALSO OTHER SUBSTANCES

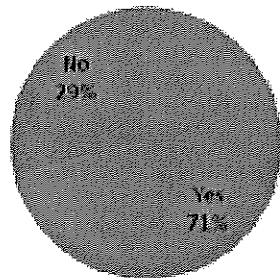
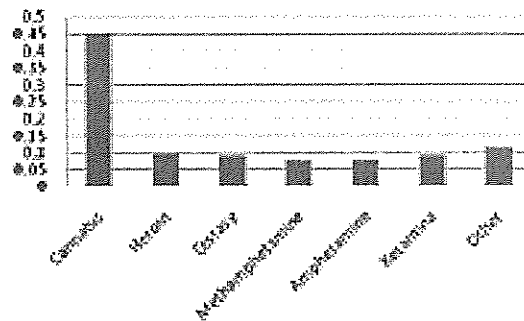


FIGURE 1D

FREQUENCY OF DEALING THE OTHER SUBSTANCES TOGETHER WITH COCAINE



References

- [1] Mozayani A. Ketamine - Effects on Human Performance and Behavior. *Forens Sci Rev* 2002;14(1/2):123-31.
- [2] Weiner AL, Vierira L, McKay CA Jr., Bayer MJ. Ketamine abusers presenting to the Emergency Department: A series of cases. *J Emerg Med* 2000;18(4):447-51.
- [3] Adams VHA. The mechanisms of action of ketamine. *Anaesthes Reanim* 1998;23(3):60-3.
- [4] Clements JA, Nimo WS, Grant IS. Bioavailability, pharmacokinetics and analgesic activity of ketamine in humans. *J Pharm Sci* 1982;71(5):539-42.
- [5] Curran HV, Morgan CA. Cognitive, dissociative and psychotogenic effects of ketamine in recreational users on the night of drug use and 3 days later. *Addiction* 2000;95(4):575-90.
- [6] Fabi, F., Ricci, R., & Rossi, C. (2011). Segmentazione e valutazione del mercato dal lato della domanda. In G. M. Rey, C. Rossi, & A. Zuliani (Eds), *Il mercato delle droghe: dimensione, protagonisti, politiche*. (pp 190-215). Venice: Marsilio editori
- [7] Fabi, F., Mammone, A. & Rossi, C. (2013). New indicators of illegal drug use to compare drug user populations for policy evaluation, *Epidemiology, Biostatistics and Public Health*, in press.
- [8] Earleywine, M., & Newcomb, M. D. (1997). Concurrent versus simultaneous polydrug use: prevalence, correlates, discriminant validity, and prospective effects on health outcomes. *Experimental and Clinical Psychopharmacology*, Nov;5(4):353-64.
- [9] Rossi C. & Mascioli F. (2014), Useful supply indicators for evaluating law enforcement efficiency, the size of the drug market and other issues, *ISSDP* 2014.
- [10] Verstraete, A.G. (2004). Detection Times of Drugs of Abuse in Blood, Urine, and Oral Fluid. *Ther Drug Monit*, 26(2):200-205.

- [11] Cone EJ, Menchen SL, Paul BD, et al. Validity testing of commercial urine cocaine metabo-lite assays: I. Assay detection times, individual excretion patterns, and kinetics after cocaine administration to humans. *J Forensic Sci.* 1989;34:15-31.
- [12] Reichman OS, Otto RA. Effect of intranasal cocaine on the urine drug screen for benzoylec-gonine. *Otolaryngol Head Neck Surg.* 1992;106:223-225.
- [13] Reiter A, Hake J, Meissner C, et al. Time of drug elimination in chronic drug abusers. Case study of 52 patients in a "low-step" detoxification ward. *Forensic Sci Int.* 2001;119:248-253
- [14] Huestis MA, Mitchell JM, Cone EJ. Urinary excretion profiles of 11-nor-9-carboxy-delta 9-tetrahydrocannabinol in humans after single smoked doses of marijuana. *J Anal Toxicol.* 1996;20:441-452.
- [15] Lafolie P, Beck O, Blennow G, et al. Importance of creatinine analyses of urine when screening for abused drugs. *Clin Chem.* 1991;37:1927-1931.
- [16] Lagravinese G.M., Mineo F., De Vita M.. Aspetti epidemiologici e considerazioni sull'affidabilità dei test tossicologici di screening eseguiti su campioni a valenza medico-legale, presso l'A.O. San Camillo-Forlanini.-Antidotes in Depth 2013- Clinical Toxicology, Substances of Abuse and Chemical Emergencies- ppg 48-49.
- [17] Kielland KB. Urinutskillelse av cannabismetabolitter [Urinary excretion of cannabis metabo-lites]. *Tidsskr Nor Laegeforen.* 1992;112:1585-1586.
- [18] Smith-Kielland A, Skuterud B, Morland J. Urinary excretion of 11-nor-9-carboxy-delta9-tetrahydrocannabinol and cannabinoids in frequent and infrequent drug users. *J Anal Toxicol.* 1999;23:323-332
- [19] Ralph Hingson, Sc.D., and Michael Winter, M.P.H. Epidemiology and Consequences of Drinking and Driving. *Alcohol Research and Health*, 2003 - pubs.niaaa.nih.gov
- [20] Starmer, G.A. Effects of low to moderate doses of ethanol on human driving-related performance. In: Crow, K.E., and Batt, R.D., eds. *Human Metabolism of Alcohol. Vol. I. Pharmacokinetics, Medicolegal Aspects, and General Interests.* Boca Ranton, FL:CRC Press,1989.pp. 101-130.
- [21] Huang MK, Liu C, Li JH, et al. Quantitative detection of ketamine, norketamine and dehydronorketamine in urine using chemical derivatization followed by gas chromatography-mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 2005; 820: 165-73
- [22] KE Moeller, KC Lee, JC Kissack. Urine drug screening: practical guide for clinicians- Mayo Clinic Proceedings, 2008 - Elsevier
- [23] T CHANG, AJ GLAZKO. Biotransformation and disposition of ketamine - International an-esthesiology clinics, 1974 International Anesthesiology Clinics:Summer 1974 - Volume 12 - Issue 2 - ppg 157-178.

