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Preliminary assessment of fentanyl and synthetic opioids prevalence among addiction patients by means of hair analysis

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ABSTRACT

Background: Although the diffusion of novel synthetic opioids has become a worldwide phenomenon, their prevalence of use in Italy seems to be limited. Existing national data is mainly derived by anamnestic surveyslacking of toxicological validation and not always disclosing the use of these compounds, which might remain underdiagnosed.

Methods: an assessment of the metabolites of the main synthetic opioids on hair samples was carried out among patients admitted at the Addiction Treatment Unit of Trento. The analytical approach included: (a) screening by means of immunoenzymatic method for fentanyl, fentanyl analogs and oxicodone; (b) confirmation of the samples resulted positive for fentanyl and oxicodone by means of HPLC-MS/; (c) search and dosage detection of Tramadol by means of HPLC-MS/MS.

Results: 3 out of 309 analysed samples were found positive: one was positive to Fentanyl and two to 4-ANPP. In the same cohort, 6 samples were also found positive for Oxycodone . Tramadol was searched in 189 samples and 12 of them resulted positive.

Discussion and conclusion: Those found positive were mainly young adults engaging in dangerous patterns of use and lacking awareness of risks. The phenomenon requires further consideration by health professionals. Training and more evidence-based information on synthetic opioids as well as other Novel Psychoactive Substances (NPS) are urgently needed.

Introduction

In recent years, the use of synthetic opioids has largely increased among the general population (Raithelhuber, 2017). The rapid and progressive spread of this phenomenon since the mid-to late 1990s can be mostly attributed to two distinct elements. The first one to consider is a general and increasing use of these molecules with highly addictive potential in clinical settings, including their prescriptions for the treatment of acute and, most of all, chronic, persistent and severe pain (Phillips, 2017). The latter often underestimate the risks of misuse, dependence and addiction.

The second relevant element about the spread of the use of synthetic opioids is due to its recreational use. These highly potent molecules are easily available at low costs and its consumption can represent a less stigmatizing condition to the user compared to other illegal drugs (Kuczyńska et al. 2018; Lovrecic et al. 2019). Despite prescription opioids are regularly used in clinical settings for the treatment of chronic pain, among other conditions, they may expose users to serious health threats. As part of this phenomenon, an exponential increment in the use of fentanyl and its derivatives has been documented at the global level (United Nations Office on Drugs and Crime, 2017). Fentanyl, a clinically well-known agent to treat chronic pain and for anesthesia, is a very

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potent opioid approved for human use. Its prescription has been regulated internationally since 1964. Since then, a large number of highly potent derivatives have emerged in the licit and illicit drug market (e.g. alfentanyl, sulfentanyl, carfentanil), exposing users to considerable health risks. A small amount of these drugs is enough to cause overdoses and much larger doses of Naloxone, than those required in case of heroin overdoses, are necessary to reverse this condition. Therefore, episodes of overdose are often fatal (Mohr et al., 2016; Armenian et al., 2018; Moore et al., 2008).

Fentanyl and derivates are often synthesized in clandestine laboratories and sold illicitly either on street or online on illegal, recreational drug markets. As a result, severe adverse outcomes, including overdose mortality have been reported across the Europe (Ciccarone, 2019; Zoorob, 2019; Dai et al., 2019) and a few cases of intoxication and death have also been reported in Italy prompting a change in legislation (Gerace et al., 2018; Dipartimento Politiche Antidroga, 2021).

The aim of this study was to evaluate the prevalence of use of popular synthetic opioids (fentanyl, oxycodone and tramadol) among a cohort of patients in treatment at the Addiction Treatment Unit of Trento.

We investigated the presence of the main synthetic opioids in hair samples collected from a number of patients in treatment for addiction. Screening was undertaken for fentanyl, oxycodone and tramadol. While fentanyl was chosen to gain a better understanding of its diffusion in the national territory, tramadol and oxycodone were chosen because some patients from our Unit declared in clinical interviews that they had used them for analgesic purposes.

Materials and methods

Study sample

All tested subjects were in treatment for addiction, as per criteria described in the Diagnostic and Statistical Manual of Mental Disorders (DSM–5). All samples were obtained as part of the routinary toxicological screening, for which all participants gave specific consent. The time frame considered was 3 months (1st December 2018-1st March 2019). Exclusion criteria included absence of head hair, recent cosmetic treatment, recent (one month) hair test, discontinuity of interaction with the Trento Addiction Unit during the three-month trial. The mean age of the tested population was 39.6 years, with a ratio male: female= 6:1. All samples were analyzed in their entire length, up to a maximum of the proximal 3 cm, since the study aimed at exploring the intake of drugs in the three months prior the collection.

Chemicals and reagents

Certified standards (1 mg/mL) of fentanyl, fentanyl-D5, tramadol and oxycodone were supplied by LGC Standards (Milan, Italy). A mixture of diluted secondary standards (0.50 mg/L) of fentanyl metabolites and analogs (norfentanyl, acetylfentanyl, furanyl-ethilfentanyl, sufentanyl, carfentanyl, alfentanyl, carboxy- valerylfentanyl, acetyl-norfentanyl, *trans*-3-methyl-norfentanyl, *cis*-3-methyl-norfentanyl, methoxy-acetyl-norfentanyl, furanyl-norfentanyl, butyryl-norfentanyl, despropionil-*para*-fluorofentanyl, carboxy-butyrylfentanyl, phenyl-acetylfentanyl, 4-ANPP, butyryl-fentanyl, beta-hydroxy-thiofentanyl) were supplied by Comedical (Trento, Italy). The Thermo-Scientific DRI immunometry reagents for fentanyl and oxycodone were distributed by Instrumentation Laboratory S.p.A - Werfen (Milan, Italy). VMA-T reagent, SLV-VMA-T wash solution, VMA-T KTZ calibrators, CTRL VMA-T and KTZ negative control, M3 reagent (R1 and R2), were provided by Comedical (Trento, Italy). The VMA-T FENT calibrator and control were prepared in the laboratory.

All samples underwent routinary screening for common drugs of abuse (methods not shown) and synthetic opioids (described as follows).

Screening test for fentanyl and oxycodone

309 hair samples, coming from the Addiction Centers from the Province of Trento (north of Italy), were collected and sent to the laboratory for routine investigations and clinical monitoring. For each sample, 33mg of hair were finely chopped and washed with 1 ml of washing solution SLV-VMA-T which was then aspirated and eliminated. Subsequently, 400 μL of VMA-T reagent was added to the samples; the tubes, hermetically sealed with a screw cap, were incubated at 100°C for one hour (Zoorob, 2019; De La Torre et al., 2010; Pichini et al., 2014). After cooling at room temperature, the liquid extract was transferred in a cup and analyzed with a DRI immunometric screening test. During the test for fentanyl, 25 mg of the extracted sample is reacted with 80 μl of R1 and 80 μl of R2. The reading was at the wavelength of 340 nm. During the oxicodone test, 13 μl of extracted sample is reacted with 90 μl of R1 and 90 μl of R2.

The analyses were performed on ILab-Taurus multiparametric analyzer supplied by Instrumentation Laboratory S.p.A - Werfen (Milan, Italy). The immunometric methods were calibrated using the CAL VMA-T-FENT calibrators (fentanyl 0,000-0,015-0,030-0,060 ng / mg) and CAL VMA-T-KTZ (oxycodone 0.00-0.25-0.50-1.00-2.00 ng / mg). At each analytical session the controls CTRL VMA-T NEG (fentanyl 0.000 ng / mg - oxycodone 0.00 ng / mg), CTRL VMA-T FEN (fentanyl 0.028 ng / mg) and CTRL VMA-T KTZ (oxycodone 0.35 ng / mg) have been included.

Based on available data in the literature obtained from real synthetic opioids users (Reisfiled, 2015; Stowe et al, 2019; Salomone et al, 2019), samples with a concentration higher than 0,300 ng/mg for oxycodone and higher than 0,010 ng/mg for fentanyl were considered positive and then sent to the confirmation analysis.

Screening test for tramadol

Among the 309 samples tested for fentanyl and oxycodone, a further analysis for tramadol was possible in 189 cases. These samples were therefore analyzed with a screening test by UPLC-MS/MS, after the extraction with 100°C incubation in Reactive VMA-T, as for the other screen tests. 50 μl of the extract were diluted in a vial with 450 μl of bi-distilled water.

With the same procedure, the CAL-VMA-T KTZ calibrators (Tramadol 0.00-0.25-0.50-1.00-2.00 ng / mg) and the CTRL VMA-T NEG controls (Tramadol 0.00 ng / mg) and CTRL-VMA-T KTZ (Tramadol 0.25 ng / mg) were diluted and analyzed.

The UPLC-MS / MS analysis was conducted on the Acquity tool UPLC - XEVO TQD (Waters Corporation), with a 1.8 μm UPLC HSS C18 Acquity column, 2.1 × 150 mm.

The mobile phase is composed of Ammonium Formate 5 mM 0.3% formic acid (solvent A) and acetonitrile (solvent B), flow 0.40 mL / min. The transition considered (ESI +) for the detection of Tramadol at the retention time of 2.16 minutes is 264.2> 58.1 (quantifier fragment): cone voltage 25 V, collision energy 15 V, temperature at the source 150°C, desolvation temperature 650°C, desolvation gas 1200 l/Hz. The samples with a concentration range higher than 0,100 ng/mg were arbitrarily considered positive (Verri et al, 2015; Kintz et al, 2018; Johansen et al, 2020) and sent to a second analysis for confirmation.

UPLC-MS/MS confirmation analysis

A further quantity of the hair samples that tested positive in the immunometric screening (for fentanyl and oxycodone) and the ones that tested positive for tramadol in the LC-MS/MS screen test were submitted to confirmation analysis by means of a fully in-house validated method. Each sample was washed twice with methanol and once with diethyl ether, then dried.

25 mg of samples, calibrators and controls were added with 500 μL of M3 reagent and 10 μL of Fentanyl D5 Internal Standard (0.10 mg/L) and incubated at 100°C for one hour[7].

Table 1
Summary of screening and confirmation results.

Cut off	0,100 ng/mg	0,300 ng/mg	0,100 ng/mg
	Fentanyl	Oxycodone	Tramadol
Number of samples	309	309	189
Negative results	306	303	177
Positive results	3	6	12
% of positive results	0,97	1,94	6,35

After cooling to room temperature, 100 μL of the extract was diluted, in special vials, with 900 μL of bi-distilled water and 10 μL were injected into an instrument UPLC – MS/MS.

The analyses were performed by using a Acquity UPLC-XEVO TQD (Waters Corporation) mass spectrometer, equipped with 1.8 μm, 2.1 × 150 mm (ESI +) Acquity UPLC HSS C18 column. The calibration curve was built with a pool of negative keratin matrix samples, fortified with different certified concentrations at six different levels. Reference analytical standards and commercially available fortified samples were added as internal quality controls.

For tramadol, the transitions considered at the retention time of 2.16 minutes is 264.2> 58.1 (quantifier), cone voltage 25 V, collision energy 15 V, and 264.2> 2461 (qualifier), cone voltage 25 V, collision energy 15 V; for oxycodone the transition considered at the retention time of 1.19 minutes is 316.1> 241.2 (quantifier) and 316.1> 256.2 (qualifier), cone voltage 35 V, collision energy 30 V.

For fentanyl, the transition considered at the retention time of 2.93 minutes is 337.2> 105.2 (quantifier) and 337.2> 188.2 (qualifier), cone voltage 40 V, collision energy 35 V; for Fentanyl-D5 the transition considered at the retention time of 2.92 minutes is 342.2> 188.2 (quantifier), Voltage Cone 25 V, Collision energy 15 V [12]. In the confirmation method for fentanyl, also 4-ANPP was monitored. This compound is either a precursor or a metabolite of several fentanyl analogues. The monitored transitions were 281.2>105.0 (quantifier), cone voltage 22 V, collision energy 32 V, and 281.2>188.0 (qualifier), cone voltage 22 V, collision energy 14 V.

Results

Of the 309 samples analyzed for fentanyl and its analogs, 306 resulted negative (< 0,010 ng/mg) and 3 samples were positive: one was positive only to Fentanyl (0,071 ng/mg), while the other two showed the presence of the precursor 4-ANPP (0,012 e 0,074 ng/mg), which is considered either a metabolite or a precursor or fentanyl (Salomone et al., 2020).

When analyzed for oxycodone, the 309 samples tested negative in 303 cases (< 0,300 ng/mg) and 6 samples were confirmed as positive (range: 0,304 - 9,350 ng/mg).

Tramadol was screened in 189 samples: 177 resulted negative (< 0,100 ng/mg) and 12 resulted positive (range: 0,100 - 33,860 ng/mg). Results from screening and confirmation analyses are summarized in Table 1. A detailed summary of all positive findings for common drugs of abuse and synthetic opioids is shown in Table 2. Quite remarkably, all subjects positive to at least one among fentanyl, oxycodone and tramadol, also tested positive to methadone.

Discussion and conclusion

From a quantitative perspective, data emerging from our research do not seem to be alarming, as these molecules prevalence settles at around only 1% of our screened population. However, some considerations are needed.

Of the three patients that tested positive to fentanyl, one was positive only to this molecule, while the other two showed, respectively, presence also of tramadol and of both oxycodone and tramadol.

Table 2
Summary of all findings in hair samples positive to synthetic opioids.

Gender	Age	Synthetic opioids	Common drugs of abuse
Male	38	Fentanyl, tramadol, oxicodone	Heroin, cocaine
Male	19	Fentanyl	Heroin, THC
Male	19	Fentanyl, tramadol	Heroin, cocaine, MDMA. ketamine
Male	26	Oxycodone	Heroin, MDMA, amphetamines
Female	60	Oxycodone	Cocaine, THC
Male	26	Oxycodone	Heroin, cocaine
Male	61	Oxycodone	Cocaine, THC
Female	50	Oxycodone	Heroin, THC
Male	29	Tramadol	Heroin, cocaine, THC
Male	55	Tramadol	THC
Female	52	Tramadol	Heroin
Male	42	Tramadol	Heroin, cocaine
Male	45	Tramadol	Heroin, cocaine
Male	32	Tramadol	Heroin, cocaine
Male	34	Tramadol	Heroin, cocaine
Male	42	Tramadol	Heroin
Male	23	Tramadol	Heroin, cocaine
Male	59	Tramadol	-

On the other hand, all other samples which resulted positive to either oxycodone or tramadol resulted negative for the fentanyl and analogs. The concentrations obtained for oxycodone and tramadol are suggestive of a frequent use, while the fentanyl concentration is more likely produced by sporadic exposure to the substance. In two cases, the precursor or metabolite 4-ANPP was detected, either because fentanyl was below the LOD, or because an analog of fentanyl was ingested by the tested individual.

In most cases, the use of oxycodone and tramadol was known to our Addiction Center professionals, as the users had previously declared the use for analgesic purposes and under regular medical prescription. These patients were treated for Opioids Use Disorder (as diagnosed according to the DSM 5) for several years (average age was 44,5 yrs for oxicodone-positive patients and 40,2 yrs for tramadol-positive patients) and over time they had established a trustful relationship with our team (Table 3).

Contrarily, the three patients who resulted positive to fentanyl were very young (average age 26,3 yrs) and were admitted to the Addiction Unit of Trento only recently. The diagnosis for these users was opioid addiction, too, but their clinical courses were suggestive of multi-drugs abuse. The use of fentanyl had not been declared to the doctor, leading our team to at least three considerations: the first is that, probably, the recent contact with our Unit and the health workers did not allow a complete disclosure of users' habits, nor a proper therapeutic relationship based on trust and communication; the second is that young people tend to abuse multiple substances at the same time (Daneceau et Al. 2019; Maremmani et Al. 2009; Bersani et Al., 2013) underestimating the possible risks; the third one is that fentanyl and its derivatives are becoming to be commonly used especially among young people for recreational purposes.

A fourth, possible explanation, is that the subjects were not aware of having taken fentanyl. This scenario is quite realistic since street substances are possibly replaced with counterfeit products.

The positivity of the samples was communicated to the interested patients: a common tendency to minimize the risks and to overestimate their ability to control the use of this drugs emerged. Unlike older patients, who tend to distrust new molecules and be cautious towards new trends, young users also showed a propensity to "experiment" with new substances, even without fully knowing their properties and associated risks.

These considerations disclose an alarming scenario: although, based on our sample, synthetic and pharmaceutical opioids use seems limited in numbers, the dangerous patterns of use and the lack of awareness among young patients requires serious consideration by health professional.

Table 3
Information about patients.

Gender	Age	Diagnosis	Scholarity	Pharmacotherapy At the time of evaluation	Duration of drug abuse	Comorbidity	Familiarity with drug abuse
Male	38	Opioids (heroine) Use Disorder	Graduation	Methadone	5 years	NO	NO
Male	19	Opioids (heroine) Use Disorder	Middle School Diploma	Methadone	1 year	NO	NO
Male	19	Opioids (heroine) Use Disorder	Middle School Diploma	No pharmacotherapy	1 year	Generalized Anxiety Disorder	Alcohol Use Disorder (father)
Male	26	Opioids (heroine)Use Disorder	High School Diploma	Methadone	3 years	NO	Opioid (heroine) Use Disorder (brother)
Female	60	Opioids (heroine) Use Disorder	Middle School Diploma	Venlafaxine Methadone	40 years	Major Depressive Disorder	NO
Male	26	Opioids (heroine) and Alcohol Use Disorder	Middle School Diploma	Methadone	1 year	NO	NO
Male	61	Opioids (heroine) Use Disorder	Middle School Diploma	Methadone	43 years	NO	NO
Female	50	Opioids (heroine) Use Disorder	High School Diploma	Methadone	12 years	Major Depressive Disorder	NO
Male	29	Opioids (heroine) Use Disorder	High School Diploma	Methadone	3 years	NO	NO
Male	55	Opioids (heroine) Use Disorder	Primary School Diploma	Methadone Quetiapine	28 years	Bipolar Disorder type 2	Gambling Disorder (father) and Alcohol Use Disorder (mother)
Female	52	Opioids (heroine) and Alcohol Use Disorder	High School Diploma	Methadone Escitalopram Zolpidem	20 years	Major Depressive Disorder	NO
Male	42	Opioids (heroine) Alcohol Use Disorder	High School Diploma	Methadone Bromazepam	5 years	Generalized Anxiety Disorder	NO
Male	45	Opioids(heroine) Use Disorder	Middle School Diploma	Buprenorphine	9 years	NO	NO
Male	32	Opioids (heroine) Use Disorder	Middle School Diploma	Methadone	3 years	NO	NO
Male	34	Opioids (heroine) and Cocaine Use Disorder	Middle School Diploma	Methadone	18 years	NO	NO
Male	42	Opioids (heroine) and Alcohol Use Disorder, Gambling disorder,	High School Diploma	Methadone Mirtazapine	8 years	Major Depressive Disorder	Alcohol Use Disorder (father)
Male	23	Opioids (heroine) Use Disorder	Middle School Diploma	Methadone	1 year	NO	NO
Male	59	Opioids (heroine) Use Disorder	Middle School Diploma	Methadone	26 years	Major Depressive Disorder	NO

In fact, the opioid abuse epidemic often stems from over-prescribing these drugs in chronic pain by physicians without considering the related risks. Since opioid analgesics can be addictive, the physicians must give the patients all the information about the potential for abuse. Opioid analgesics shouldn't be taken for longer than 3 to 6 months to avoid the risk of iatrogenic addiction. General Practitioners are sometimes unaware of this phenomenon, for this reason, we believe that sharing with colleagues is essential to reduce the spread of this epidemic.

Legally opioid analgesics are only available by prescription of a doctor, however in recent years the abuse of these drugs have increased primarily through two means: the illegal online sales on the black market and a method known as "diversion", when the drugs, prescribed legally, are then passed or sold on to third party users. About 4% of the adult US population misuses prescription opioids, and in 2015, more than 33,000 deaths were attributable to overdose with licit and illicit opioids (Skolnick, 2018).

Unauthorized online pharmacies sell drugs even without prescriptions. These drugs are very often counterfeit, produced by clandestine laboratories, contaminated by added substances and are therefore dangerous and often deadly.

More information, better communication, and accurate sharing of news on Fentanyl, its derivatives and in general on all Novel Psychoactive Substances are needed. Novel Psychoactive Substances can be definite as a new narcotic or psychotropic drug, in pure form or in preparation, that is not controlled by the United Nations drug conventions, but which may pose a public health threat as other illegal Substances (EMCDDA, 2021).

Information must be shared not only between health professionals and users, but also among specialist services, hospitals and general practitioners, who are often the first prescribers of opioid analgesics or the first contacts for users developing an Opioid Use Disorder. Further attention should be paid to illicit manufactures and selling sites, in order to prevent the spread of the severe social and health consequences linked to such use. This is certainly a great challenge for the future, in which clinicians and researchers must commit and believe.

Author statement

Ermelinda Levari carried out the literature review, contributed to subject recruitment and wrote the first draft of the manuscript;

Andrea Lotti performed the laboratory analysis and collaborated to the preparation of this work;

Marinella Frenguelli advised on the laboratory and statistical analysis;

Valentina Longo collaborated to the statistical analysis and to the laboratory analysis;

Massimo Gottardi collaborated to the laboratory analysis;

Giovanni Michele Marchio collaborated to the laboratory analysis;

Martina Stefani worked on the preparation of this manuscript;

Roberta Ferrucci revised this manuscript at the first stage;

Attilio Negri contributed to the data analysis and the preparation and submission of this work;

Ornella Corazza advised on the design of the study and supervised the overall data collection, analysis and preparation of this manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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