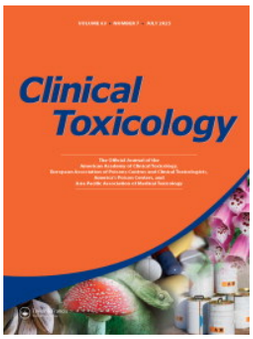

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CLINICAL RESEARCH

Xylazine detection in urine of fentanyl-positive patients from a single academic center

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ABSTRACT

Introduction: Xylazine has increasingly been used as an additive in illicit drugs, leading to severe health consequences. The current study aims to define the total xylazine-positive cases since 2010 and to analyze the trends and clinical implications of xylazine-positive cases at the University of Pittsburgh Medical Center from 2020 to 2024.

Methods: This cross-sectional academic laboratory-based study analyzed the mass spectrometry dataset, including the normalized peak sizes of xylazine and fentanyl in xylazine-positive urine comprehensive drug screening cases, along with their clinical information. The laboratory information system was also queried to obtain the total number of xylazine detections in urine comprehensive drug screening since 2010.

Results: A total of 351 xylazine-positive adult cases in urine comprehensive drug screening were examined to identify trends in urine xylazine and fentanyl relative concentrations between 1 April 2020 and 31 March 2024. After excluding outpatient cases, the urine xylazine and fentanyl relative concentrations were also correlated with clinical features for the remaining 249 cases. Xylazine-positive cases have increased since 2016, with a sharp rise between 2019 and 2021. Urine xylazine relative concentrations showed a minimal decline, while urine fentanyl relative concentrations modestly decreased. Patients with skin wounds and infections, but not with coma or post-cardiac arrest, had significantly higher urine xylazine relative concentrations than the entire cohort.

Discussion: The number of xylazine-positive and fentanyl-positive cases has increased over time, even as the relative concentrations of urine xylazine and fentanyl have gradually declined. This inverse trend suggests an increasing prevalence of xylazine and fentanyl exposure at progressively lower concentrations. Our data also indicated the association of urine xylazine concentrations with skin wounds and infections, but not with coma or post-cardiac arrest.

Conclusions: The findings of this study highlight the increasing prevalence of xylazine in illicit drug use, particularly in combination with fentanyl, at our institution over time. More research is needed to elucidate the roles of xylazine on opioid intoxication.

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Adulterant; fentanyl; opioid epidemic; poisoning; xylazine

Introduction

Xylazine use has emerged as a critical public health issue [1]. Xylazine is an α_2-adrenergic agonist approved by the United States Food and Drug Administration (US FDA) as a sedative, but only for veterinary use. However, it is an adulterant in illicit drugs, primarily diacetylmorphine (heroin) and fentanyl, presumably to prolong euphoria [2] and to delay opioid withdrawal [1,3]. Studies have highlighted its harmful effects, including respiratory and central nervous system (CNS) depression, reduced cardiac output, bradycardia, and skin wounds and infections [1,4–6]. No specific human antidote is approved for human use [7,8]. Based on its pharmacological actions and animal studies [9,10], xylazine is suspected to enhance opioid effects synergistically, increasing the risk of overdose or death [6], although conflicting data exist [11–14]. Overall, it is unclear if xylazine increases the risk of death from fentanyl overdose, and thus, it is crucial to determine the pathophysiological effects of xylazine in opioid overdose cases.

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The number of xylazine-associated opioid overdoses and deaths has sharply increased recently. Xylazine use was first reported in Puerto Rico in the early 2000s and later spread to the US mainland. The Northeast region of the US has the highest rate of xylazine-related overdoses [2]. The US Centers for Disease Control and Prevention (CDC) noted a 1,238% rise in xylazine-related deaths from 2018 to 2021 [1]. In April 2023, the White House Office of National Drug Control Policy declared fentanyl mixed with xylazine a national health threat, prompting healthcare actions [1].

A vital aspect of this response is urine drug screening, which is occasionally a routine test for suspected overdose cases. However, quick immunoassays for xylazine were not clinically available in the US as of October 2024. Thus, there are limited clinical laboratory data available on xylazine. Most previous xylazine studies were conducted in the forensic domain, evaluating either opioid overdose deaths or seized illicit drugs by law enforcement. Furthermore, many coroners' and medical examiners' offices did not routinely test for xylazine [2]. The limited availability of xylazine testing in both clinical and forensic settings (especially before 2020) has resulted in an unclear true prevalence and recent trends of xylazine use in the US.

Our clinical toxicology laboratory at the University of Pittsburgh Medical Center Presbyterian Hospital has offered urine comprehensive drug screening, which is ordered by clinicians based on clinical needs, as part of routine clinical testing to the University of Pittsburgh Medical Center hospitals in the Pittsburgh area for over 20 years. Urine comprehensive drug screening employs untargeted mass spectrometry data acquisition for the qualitative detection and timely monitoring of emerging drugs, including xylazine [15,16]. We are one of the few clinical toxicology laboratories that reported xylazine in urine comprehensive drug screening reports in the early phase of the current opioid crisis. This study examines the urine comprehensive drug screening dataset to identify recent xylazine trends at our academic center in western Pennsylvania and correlate laboratory findings with the clinical features.

Methods

Study design and setting

This single laboratory-based cross-sectional study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline. This study was approved as exempt research by the University of Pittsburgh IRB (Study #24030084).

The laboratory information system was queried to obtain the total number of xylazine detections in urine comprehensive drug screening since 2010 (Figure 1). For the subsets of these xylazine-positive urine comprehensive drug screening cases as defined later, the mass spectrometry dataset containing the detected analytes and their peak sizes and associated clinical information were further analyzed to learn the trends and clinical correlations of urine xylazine and fentanyl relative concentrations.

Inclusion and exclusion criteria of xylazine-positive urine comprehensive drug screening cases

The inclusion criteria for these subset urine comprehensive drug screening cases were: xylazine-positive urine comprehensive drug screening cases determined by liquid chromatography quadrupole time-of-flight mass spectrometry signed out by one of the authors between 1 April 2020, and 31 March 2024. The exclusion criteria for the trends evaluation of urine xylazine and fentanyl relative concentrations (defined as the "Trends" cohort) were positive urine comprehensive drug screening cases determined by gas chromatography-mass spectrometry, minor cases (age <18 years), including unintentional or *in utero* xylazine exposure, duplicated cases within the same visit/encounter, and the cases with missing fentanyl peak size values. Additional exclusion criteria for the clinical correlation study (defined as the "Correlation" cohort) were the outpatient cases from opioid clinics and pain clinics, who did not display overt clinical symptoms associated with xylazine (Figure 1).

Data analysis

From the mass spectrometry dataset, normalized peak sizes of xylazine and fentanyl were calculated as surrogates of the urine concentrations (or relative urine concentrations) of these drugs by dividing the peak size values by that of the internal standard. This normalization procedure was performed to mitigate the analytical bias due to the peak intensity drift and ion suppression [17]. Xylazine to fentanyl peak size ratios were also calculated. The relative urine concentrations are unitless values and thus, these values are given in arbitrary units in this manuscript.

Outcomes

A priori primary outcomes include (1) the trends of aggregated normalized urine xylazine and fentanyl peak sizes, and (2) the clinical correlations of the aggregated normalized urine xylazine and fentanyl

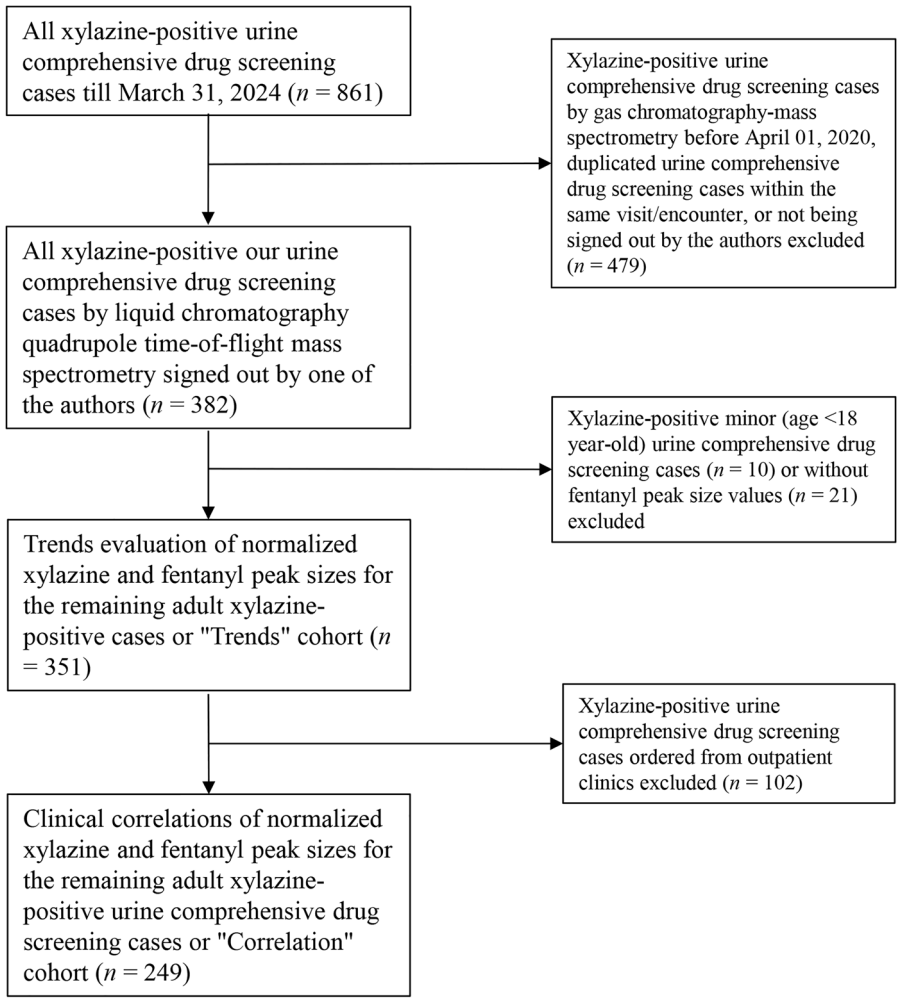

Figure 1. The flow diagram of the xylazine-positive urine comprehensive drug screening cases with inclusion/exclusion criteria.

peak size values with skin wounds and infections, coma, and post-cardiac arrest.

Trends of aggregated normalized urine xylazine and fentanyl peak sizes

The trends of aggregated normalized xylazine and fentanyl peak sizes in urine comprehensive drug screening were assessed between 1 April 2020, and 31 March 2024, for the "Trends" cohort (Figure 3). The data were tabulated quarterly and analyzed. The entire dataset was also split into two groups, a first-and-second-year group (2020 second quarter (Q2) – 2022 first quarter (Q1)) and third-and-fourth-year groups (2022 Q2 – 2024 Q1) to compare the first and second halves of the data over time.

Clinical correlation of aggregated normalized urine xylazine and fentanyl peak sizes

Clinical correlations between aggregated normalized xylazine and fentanyl peak size values in urine comprehensive drug screening and clinical features (skin wounds and infections; coma; post-cardiac arrest) were also evaluated by medical chart reviews for the "Correlation" cohort (Figure 4). Specifically, the case classification by clinical features was made manually by searching for the following words ("skin ulcer", "skin wound", "skin abscess", "skin infection", or "cellulitis") for skin wound and infection, ("unresponsiveness", "coma", or "comatose") for coma, and ("arrest" or "asystole") for post-cardiac arrest in the medical records of the clinical visits/encounters at the index admission and upon arrival to the hospital associated with the particular urine comprehensive drug screening cases. Medical record abstraction was conducted without blinding to study outcomes by the authors (pathologists), who had no previous medical record abstraction training.

The comatose patients who resulted from cardiac arrest were only considered under the post-cardiac arrest category, whereas the comatose patients who regained consciousness by therapeutic interventions (e.g., naloxone) were categorized under the comatose category. The patients with skin infection and wounds

who also had a cardiac arrest were counted under both categories.

Statistical analysis

Data distributions were visually assessed on histograms, which revealed substantial skew and deviation from normality. Based on this observation, nonparametric statistical tests were applied. The statistical analysis for two groups was made using the Wilcoxon Rank Sum test (Figure 3 and Table 2), and the statistical analysis for more than three groups was made using the Kruskal-Wallis test and Dunn's post hoc test with R (Figures 3 and 4 and Table 2). *P* values less than 0.05 were considered statistically significant.

Laboratory testing

Urine comprehensive drug screening was conducted using a dilute-and-shoot method on liquid chromatography-quadrupole time-of-flight mass spectrometry for untargeted data acquisition in positive electrospray ionization mode since 2020 [15,16]. Detailed analytical information is provided in Supplementary Text). A different mass spectrometry system (gas chromatography-mass spectrometry) was used for urine comprehensive drug screening before 1 April 2020, and thus, no comparable peak size data were available before that date.

Results

Study subjects

The data in our laboratory information system indicates that there are 861 xylazine-positive urine comprehensive drug screening cases in total as of 31 March 2024 (Figure 1). After excluding the xylazine-positive urine comprehensive drug screening cases by gas chromatography-mass spectrometry before 1 April 2020, duplicated urine comprehensive drug screening cases within the same visit/encounter, urine comprehensive drug screening cases not signed out by the authors, urine comprehensive drug screening minor cases (age <18 years), and the cases with missing fentanyl peak size values in the dataset, the remaining 351 cases of the "Trends" cohort were analyzed to evaluate the trends of normalized urine xylazine and fentanyl peak sizes between 1 April 2020 and 31 March 2024 (Figure 3). Next, after excluding 102 cases from pain and outpatient opioid clinics, the remaining 249 cases of the "Correlation" cohort were further analyzed to evaluate the clinical correlations of normalized urine xylazine and fentanyl peak sizes (Figure 4).

The demographic and clinical characteristics of these 249 adult xylazine-positive urine comprehensive drug screening cases ("Correlation" cohort) are provided in Table 1. Overall, the patients in the "Correlation" cohort were more frequently male (53.8%, 134/249), non-Hispanic white (72.7%, 181/249), and between the ages of 25 and 44 years (67.4%, 168/249). More than half of the patients were admitted to the general medical floor for further management (51.8%, 129/249). The second most common hospital stay location was the intensive care unit (34.9%, 87/249), followed by the emergency department (10.4%, 26/249). Moreover, 19.3% (48/249) of cases corresponded to patients seeking detoxification services or preoperative evaluations. Finally, 7.2% (18/249) of patients expired during

Table 1. Demographic characteristics of the clinical "Correlation" study cohort ($n = 249$).

Demographic variables	Clinical Correlation cohort ($n = 249$)[a]
Sex (%)	
Male	134 (53.8%)
Female	115 (46.2%)
Race (%)	
White, non-Hispanic	181 (72.7%)
Black, non-Hispanic	53 (21.3%)
Hispanic[b]	2 (0.8%)
Other, non-Hispanic[c]	3 (1.2%)
Unknown[d]	10
Age group, years (%)	-
Mean age (years)	40.6
<25	7 (2.8%)
25–34	88 (35.3%)
35–44	80 (32.1%)
45–54	30 (12.0%)
≥55	44 (17.7%)
Location of hospital stay (%)	
Emergency department	26 (10.4%)
General medical floor	129 (51.8%)
Intensive care unit	87 (34.9%)
Other[e]	6 (2.4%)
Unknown[f]	1
Detoxification/preoperative cases (%)	48 (19.3%)
Outcomes (%)	
Comatose/unresponsive[g]	65 (26.1%)
Cardiac arrest	17 (6.8%)
Death[h]	18 (7.2%)
Skin involvement (%)[i]	33 (13.3%)
Wounds/ulcers	14 (5.6%)
Infection (cellulitis/abscess)	24 (9.6%)

[a]Clinical correlation study cohort: 235 unique patients, 249 events analyzed (12 patients in two events and one patient in three events).
[b]Hispanic or Latino.
[c]Non-Hispanic Native American or Alaskan Native, Asian, or Native Hawaiian or Other Pacific Islander.
[d]Not disclosed in clinical chart.
[e]Outpatient or preoperative setting.
[f]No available information in clinical charts.
[g]Out of 65 patients, 35 required endotracheal intubation due to respiratory failure.
[h]Deceased patients include 14 patients admitted for post-cardiac arrest and two patients for comatose/unresponsiveness.
[i]Three cases were counted in both wounds/ulcers and infection (cellulitis/abscess) categories.

the hospital encounter where a xylazine-positive sample was recorded.

Xylazine cases and trends

Xylazine was first identified at the University of Pittsburgh Medical Center in 2016 (two cases), according to the laboratory information system, and the number of xylazine-positive cases has since increased. A sharp increase in xylazine-positive cases was also observed between 2019 (41 cases) and 2021 (208 cases) (Figure 2A). Either 6-monoacetylmorphine (a diacetylmorphine-specific metabolite) and/or fentanyl—including fentanyl analogs (fentalogs) or their metabolites—were co-detected in all xylazine-positive urine comprehensive drug screening cases. A sharp increase in 6-monoacetylmorphine- and fentanyl-positive cases was also observed between 2015 (four cases and zero cases, respectively) and 2018 (178 cases and 1,137 cases, respectively) (Figure 2B).

The urine xylazine relative concentrations displayed a minimal elevation between the 2020 Q2 and the 2024 Q1 ($P=0.02$) and between the 2020 Q3 and the 2024 Q1 ($P=0.01$), but a minimal decline in biannually tabulated data ($P=0.01$) in the "Trends" cohort (Figure 3A and Table 2). In contrast, urine fentanyl relative concentrations showed a modest decrease in biannually tabulated data ($P<0.001$) but not in quarterly tabulated data (Figure 3B and Table 2). The ratio of xylazine to fentanyl relative concentrations were more pronounced increase between the 2020 Q2 and the 2024 Q1 ($P=0.02$), between the 2020 Q3 and the 2023

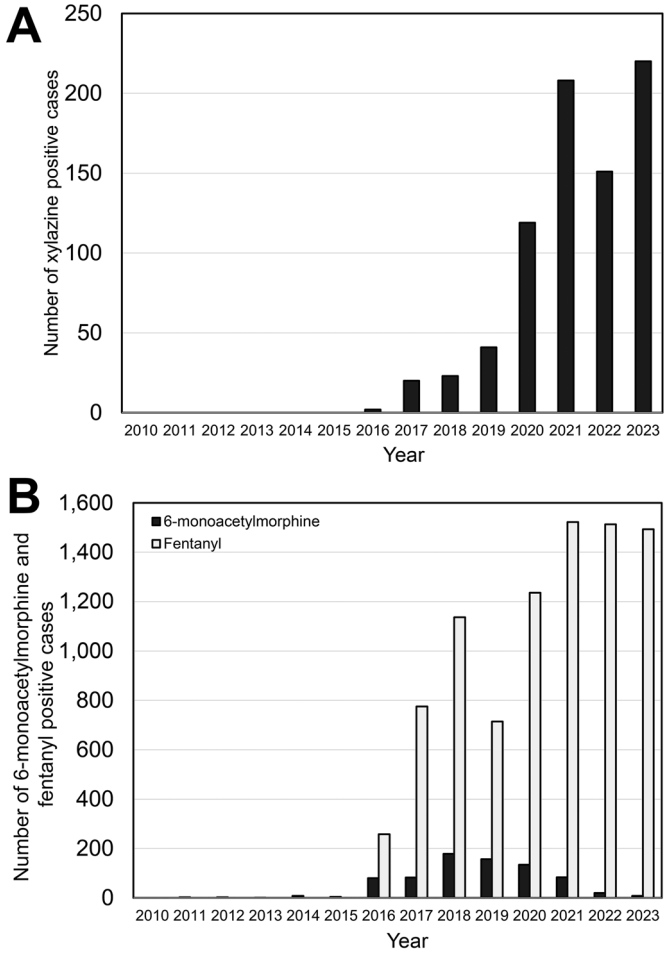

Figure 2. The number of xylazine-positive cases (A) and 6-monoacetylmorphine- and fentanyl-positive cases (B) detected by urine comprehensive drug screening at the University of Pittsburgh Medical Center since 2010. Seventy-seven xylazine-positive cases, zero 6-monoacetylmorphine-positive cases, and 174 fentanyl-positive cases in the first quarter of 2024 were not included in the figure. Gas chromatography-mass spectrometry was used until the end of the first quarter of 2020, and liquid chromatography-quadrupole time-of-flight mass spectrometry has been used for urine comprehensive drug screening since the second quarter of 2020. The lowest detection limits are approximately 50 µg/L for xylazine, 30 µg/L for 6-monoacetylmorphine, 20 µg/L for fentanyl, and 40 µg/L for norfentanyl for gas chromatography-mass spectrometry and 10 µg/L for xylazine, 20 µg/L for 6-monoacetylmorphine, 10 µg/L for fentanyl, and 20 µg/L for norfentanyl for liquid chromatography-quadrupole time-of-flight mass spectrometry, respectively.

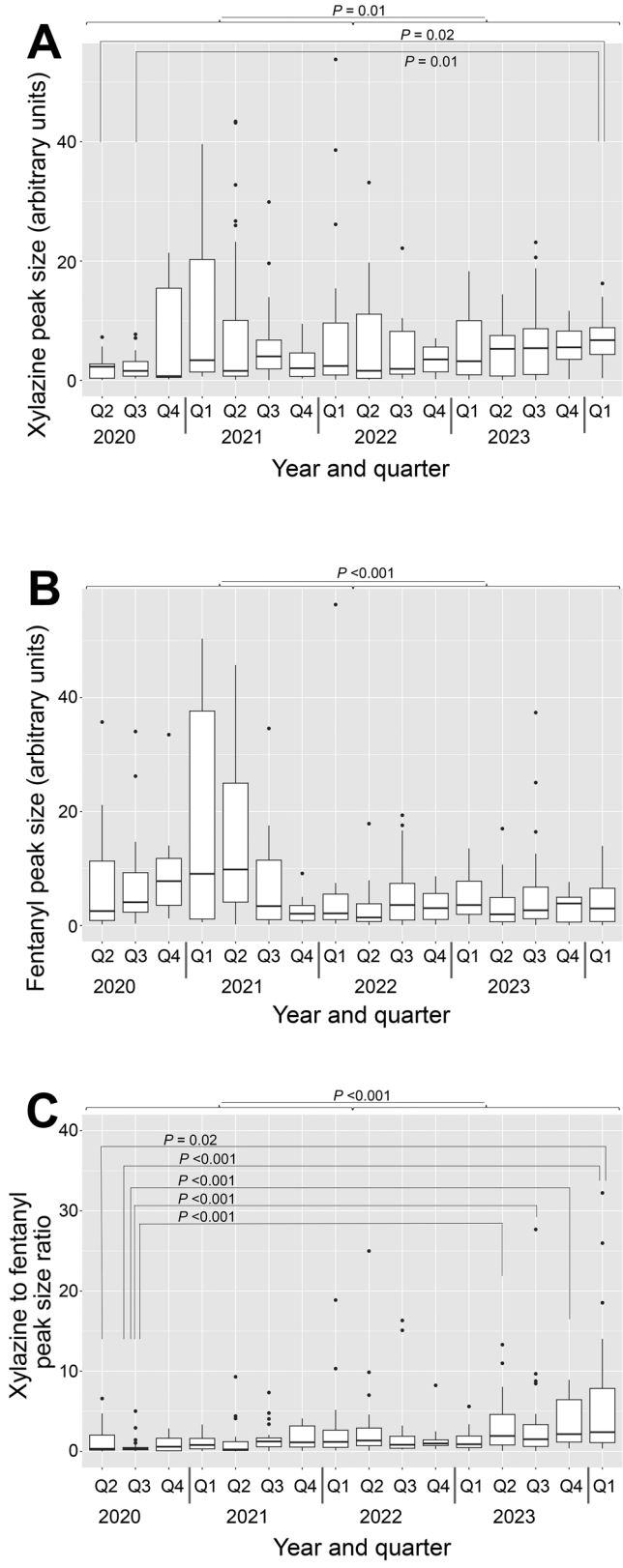

Figure 3. Box-and-whisker plots of the peak size of urine xylazine (A), fentanyl (B), and xylazine-to-fentanyl ratio (C) in urine comprehensive drug screening between 2020 Q2 and 2024 Q1. Each box represents the interquartile range, spanning from the 25th percentile to the 75th percentile. The horizontal line inside the box indicates the median value. The whiskers extend to the smallest and largest values within 1.5 times the interquartile range, respectively. Both xylazine and fentanyl peak sizes are normalized with that of internal standard. Statistical analyses (*P* values) are given within the figure. One outlier (209.64, 2024-01-03) was removed from Figure 2C.

Table 2. Summary of the pairwise-comparisons in the trends of aggregated normalized urine xylazine and fentanyl peak sizes (Figure 3) and clinical correlation of aggregated normalized urine xylazine and fentanyl peak sizes (Figure 4). "All" refers to the entire "Correlation" study cohort, "Arrest" refers to post-cardiac arrest cases, "Coma" refers to unresponsive/comatose cases, and "Skin" refers to skin wound and infection cases.

Study	Analyte(s)	Pairwise comparisons	*P* values
Trends (Figure 3A)	Xylazine	First 2-year versus last 2-year	0.01
		2020 Q2 versus 2024 Q1	0.02
		2020 Q3 versus 2024 Q1	0.01
Trends (Figure 3B)	Fentanyl	First 2-year versus last 2-year	<0.001
Trends (Figure 3C)	Xylazine to fentanyl ratio	First 2-year versus last 2-year	<0.001
		2020 Q2 versus 2024 Q1	0.02
		2020 Q3 versus 2023 Q2	<0.001
		2020 Q3 versus 2023 Q3	<0.001
		2020 Q3 versus 2024 Q1	<0.001
Clinical correlations (Figure 4A)	Xylazine	All versus Skin	0.04
		Arrest versus Skin	0.03
		Coma versus Skin	0.01

Q2 ($P<0.001$), between the 2020 Q3 and the 2023 Q3 ($P<0.001$), between the 2020 Q3 and the 2023 Q4 ($P<0.001$), between the 2020 Q3 and the 2024 Q1($P<0.001$), and between the first two years (2020 Q2-2022 Q1) and the last two years (2022 Q2-2024 Q1) ($P<0.001$) (Figure 3C and Table 2).

Xylazine and clinical features

The urine xylazine relative concentrations in cases with skin wounds and/or infections were significantly higher than in the entire "Correlation" cohort ($P=0.04$), as well as comatose cases ($P=0.01$) and post-cardiac arrest cases ($P=0.03$) (Figure 4A and Table 2). The median values of urine xylazine relative concentrations in comatose patients (2.81 arbitrary units) and patients with post-cardiac arrest (2.38 arbitrary units) were not statistically different than those in the entire "Correlation" cohort (3.56 arbitrary units) (Figure 4A). Likewise, the urine fentanyl relative concentrations and xylazine-to-fentanyl ratios were not significantly different among these groups (Figures 4B and 4C).

Discussion

Our urine comprehensive drug screening data indicate that xylazine has been detected since 2016, with a sharp increase between 2019 and 2021 in our institution. This is consistent with the National Forensic Laboratory Information System and US CDC data demonstrating the increase of xylazine involvement among the forensic laboratory data across the US between 2019 and 2022 [18,19]. Additionally, it is consistent with the recent trends of xylazine-related overdose deaths reported by other authors [20,21]. While the study was conducted in a specific academic medical center laboratory, both symptomatic as well as asymptomatic xylazine-positive patients were included. Thus, the study findings should reflect the more general xylazine-exposed patient populations and can be extrapolated to similar urban settings as well.

Our urine comprehensive drug screening data also indicates a prominent increase in the urine xylazine-to-fentanyl ratios between 2020 and 2024, to which the modest decrease in urine fentanyl relative concentrations contributes. Urine fentanyl relative concentrations would also correlate with a slight decrease in overdose deaths between 2021 and 2023 in Allegheny County in western Pennsylvania (718 in 2021, 693 in 2022, and 668 [preliminary] in 2023) [22]. Similarly, opioid overdose-related emergency department visits have been decreasing since 2021 in Allegheny County (2.16 per 10,000 in 2021 Q3, 1.67 per 10,000 population in 2023 Q3, and 1.07 per 10,000 population in 2024 Q1) [22]. Nevertheless, these changes in the relative concentrations of urine fentanyl and xylazine between years and quarters are relatively small, and the clinical utility of these analyses might be limited.

Our data shows that the cases with skin findings have significantly higher urine xylazine relative concentrations than the overall "Correlation" cohort, comatose cases, and post-cardiac arrest cases. Both skin wounds and infections are common complications of illicit drug injections [23] and associated with the development of skin ulcers [24]. Nevertheless, these distinctions are not always clear in the medical chart review, and thus, selection and documentation biases might have been introduced if we had attempted to count xylazine-characteristic skin wounds and ulcers. Instead, we counted the cases with skin wounds and infections, which should include xylazine-characteristic skin wounds, in addition to skin conditions secondary to illicit drug injections and other etiologies. Interestingly, the urine fentanyl relative concentrations in the skin wound and/or infection cases were not different from the ones in the overall "Correlation" study cohort, comatose cases, and post-cardiac arrest cases.

The effects of xylazine on opioid intoxication are controversial. As a sympatholytic drug, xylazine has

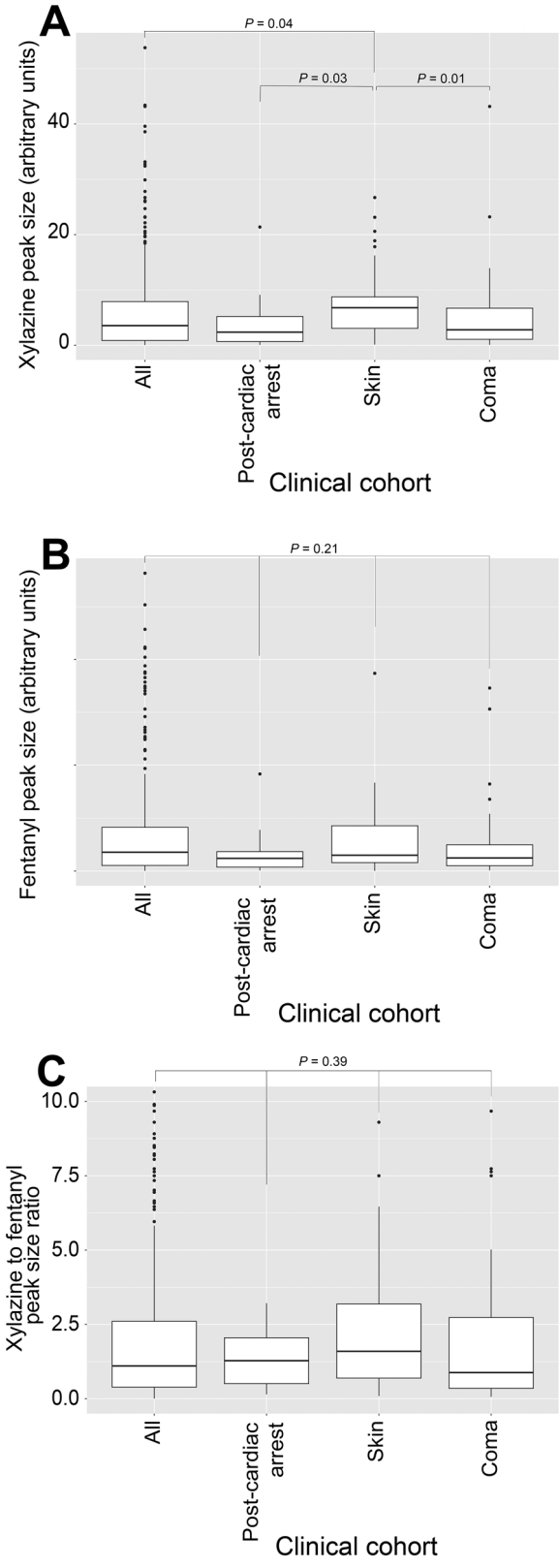

Figure 4. Box-and-whisker plots of urine xylazine relative concentrations (A), urine fentanyl relative concentrations (B), and xylazine-to-fentanyl ratios (C) in different clinical groups. Each box represents the interquartile range, spanning from the 25th percentile to the 75th percentile. The horizontal line inside the box indicates the median value. The whiskers extend to the smallest and largest values within 1.5 times the interquartile range, respectively. The groups include the entire (All) "Correlation" study cohort, post-cardiac arrest cases (Post-cardiac arrest), skin wound and infection cases (Skin), and unresponsive/comatose cases (Coma). The urine xylazine relative concentrations in cases with skin ulcers and chronic wounds are significantly higher than in the entire study cohort, as well as unresponsive/comatose cases and post-cardiac arrest cases. Outliers above 10.5 (twelve outliers in the entire (All) "Correlation" study cohort and one outlier in the skin wound and infection cases) were removed from Figure 3C.

sedative effects, which may have synergistic effects when combined with opioids. That is why serious health concerns have been raised about xylazine as a possible potentiator of opioid intoxication [1,6]. Consistently, xylazine has been shown to potentiate opioid toxicity in animal studies. In a rat study, xylazine was shown to exacerbate fentanyl-induced brain hypoxia by attenuating the post-hypoxic compensatory increase of brain oxygenation after the initial respiratory suppression by fentanyl [25]. A recent preprint article of a mice study showed that xylazine had no effect on fentanyl-induced hypoxia but acted mainly as an exacerbator of fentanyl-associated bradypnea by increasing the expiration time [26]. However, contradictory reports have also been published. A recent multicenter prospective clinical study showed that xylazine adulteration of illicit opioids is associated with decreased odds of cardiac arrest and coma as compared to illicit opioids without xylazine adulteration among opioid-overdosed patients [13]. In another recent forensic study of lethal opioid overdose cases, postmortem fentanyl concentrations were higher in cases with xylazine than in the ones without xylazine [11]. Xylazine was recently shown to be a full agonist of the κ-opioid receptor [27], which should antagonize respiratory depression through the μ-opioid receptor [28,29]. Overall, conflicting data exist regarding the role of xylazine in opioid poisoning.

In our study, comatose and post-cardiac arrest patients did not have significantly higher urine xylazine relative concentrations than the entire "Correlation" study group. Overall, the aggregated urine xylazine concentrations are more associated with skin findings, but not with coma and post-cardiac arrest, in our study.

Limitations

This study had several limitations. First, urine drug concentrations may not correlate well with blood drug concentrations in each patient. Nevertheless, the aggregated urine drug relative concentrations should still reflect the overall trends of the drug of the cohort. In other words, it is reasonable to speculate that the cohort with higher urine xylazine relative concentrations would have been exposed to more xylazine than the other cohort with lower urine xylazine relative concentrations. The aggregated urine drug concentrations were also used to estimate the amounts of previous drug exposures of the study cohorts in an epidemiological study before [30].

One might argue against urine peak size analysis of fentanyl and xylazine without peak size correction by urine creatinine concentrations. The dilute-and-shoot method is also subject to matrix effects, particularly ion suppression, in liquid chromatography-mass spectrometry analysis [16]. However, liquid chromatography-mass spectrometry analysis in the positive electrospray ionization mode is less prone to ion suppression than in the negative electrospray ionization mode for urine specimens [31]. Our experience also suggests that the peak size comparisons in the positive electrospray ionization mode provide reasonable estimates of the compound concentrations in most cases. The normalization of peak size with that of internal standards should further mitigate the bias.

Another limitation includes the exclusion of fentanyl metabolites and other opioids including fentalogs and 6-monoacetylmorphine in this study. This could explain why the urine fentanyl relative concentrations of unresponsive/comatose and post-cardiac arrest cases were not higher than those of the entire "Correlation" cohort. A recent report shows that the half-life of fentanyl is shorter than that of xylazine [32]; thus, fentanyl disappears quicker than xylazine. But we did not evaluate fentanyl metabolites including norfentanyl in this study. Similarly, we did not assess fentanyl analogs and their metabolites, even though fentanyl analogs are frequently included in the current illicit fentanyl supplies [33–35]. These limitations could explain the apparent lack of difference in the urine fentanyl relative concentrations among these groups.

Another potential confounder is iatrogenic fentanyl, which might be given to alleviate discomfort for the intubated patients, even in an opioid-overdose situation. Some fentanyl-positive cases were iatrogenic. Small parts of the fentanyl peak sizes might be attributable to iatrogenic cases, especially the unresponsive/comatose and post-cardiac arrest cases. But the amounts of medically administered fentanyl should be much less than the illicit ones, and the medically administered fentanyl should not alter the results of clinical correlation of aggregated normalized urine fentanyl peak sizes.

Exclusion of metamfetamine in this study would be another limitation. Fentanyl is often co-mixed with street metamfetamine [36], and if vasoconstriction is the mechanism by which ischemia occurs that causes skin ulcers, the presence or absence of methamfetamine and any other α-adrenergic agonists can be an important confounder.

Other possible biases include lack of blinding of chart abstractor to study outcomes and selection bias during the medical record abstraction. Even though the primary outcomes were assessed carefully, some aspects were not entirely objective. For example, objective mental status parameters, such as the Glasgow Coma Scale, were not reviewed during the

medical chart review. Nevertheless, every effort was made to ensure that the abstraction process was thorough and consistent.

Even with these limitations, we believe this study offers many new perspectives. The population studied is not as frequently included in the literature, as most studies focus on fatalities and do not routinely consider non-fatal overdoses and stable outpatient cases. This could be related to the availability of testing for xylazine in clinical settings. In addition, the large clinical cohort allowed us to evaluate different clinical effects associated with xylazine, besides exploring controversial topics that are not fully understood.

Conclusions

The findings of this study underscore the increasing prevalence of xylazine in illicit drug use, particularly in combination with fentanyl, at our institution from 2016 to 2024. Notably, patients with skin ulcers and chronic wounds, but not with coma or post-cardiac arrest, had higher urine xylazine relative concentrations than the entire cohort, indicating a possible association of urine xylazine relative concentrations with skin wounds and infections, but not with coma and post-cardiac arrest. Currently, there are conflicting data regarding the roles of xylazine on opioid poisoning. More research is needed to elucidate the roles of xylazine on opioid intoxication. Specifically, prospective clinical studies with both urine and blood xylazine concentrations are needed to address the knowledge gap for xylazine.

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AI used in manuscript preparation

Microsoft CoPilot was used for manuscript editing and improvement to enhance the clarity of the content.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Data availability statement

The original datasets generated and/or analyzed during the current study contain protected health information (PHI); thus, cannot be publicly shared due to the Health Insurance Portability and Accountability Act (HIPAA) Privacy Rule in the U.S. The de-identified data analyzed during this study are included in this published article.

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