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RESEARCH ARTICLE

Comparison of urine trace element levels in tramadol addiction alone and its co-abuse with cigarette and opium in Western Iran

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Abstract

Tramadol is an opioid pain medication used to treat moderate to severe pain. Tramadol consumers tend to co-abuse some other substances such as opium, cigarettes, alcohol, and cannabis and each of these substances may impair trace elements homeostasis in the body. Therefore, this case–control study aimed to compare the urinary concentration of some essential and toxic elements in tramadol addiction alone and its co-abuse with cigarette and opium in Western Iran. For this purpose, urine samples were collected in two groups of tramadol ($n=72$) and control subjects ($n=62$) from March to November 2020. The case group was divided into three groups: tramadol alone, tramadol + opium, and tramadol + cigarettes. Moreover, ICP-MS (Agilent 7900) was used to measure trace element concentrations in the urine samples. Based on our results, Fe was the only element markedly higher among controls as compared to tramadol users ($p<0.001$). Moreover, the concentration levels of As appeared to be the same among both groups, but the levels of other elements including Ca, Cd, Cr, Mn, Cu, Zn, Co, Ni, Se, and Pb were all significantly higher among tramadol users as compared to control group. The rank-based regression analysis illustrated that no contribution of sex and age effect was found by the regression model on the levels of all 12 studied elements. While, smoking was found to affect the levels of Fe ($\beta=0.163$, $P=0.025$) and Co ($\beta=0.411$, $p<0.001$) so that smoking reduced Fe levels but elevated Co concentration levels. Abuse of tramadol along with cigarettes and opium increased the concentration of some heavy metals in urine samples compared to the control group. However, these results showed no significant effect of age, sex, smoking habit, and amount of tramadol usage on the levels of trace elements.

Keywords Trace elements · Opium dependence · Smoking · Tramadol · Regression analysis

Introduction

Drug and quasi-drug use has become a global health problem. This leads to numerous consequences at the health, economic, social, and legal levels in society (Alinejad et al. 2018; Ansari-Moghaddam et al. 2012, 2016). Tramadol is a centrally-acting analgesic medication that is used to relieve moderate to severe pain. It activates the opioidergic and serotonergic systems in the central nervous system (CNS) to reduce pain (Nakhaee et al. 2021b). The use of this drug is widespread today and in many cases is associated with poisoning due to its use (Ghane et al. 2018; Masjedi et al. 2013). Increasing the dose of tramadol along with other drugs can threaten people's health and in severe cases lead to death (Nakhaee et al. 2021b).

Abuse of these substances can also affect the absorption of essential metals in the body such as zinc, calcium, copper, and iron and upset the balance of these metals essential for biological activities (El-Safty et al. 2018; Mehrpour et al. 2012). Essential metals play an active role in metabolic activity by interacting with biological molecules and regulating many cellular metabolic reactions (Fraga 2005, Osredkar and Sustar, 2011). They also play an important

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role as antioxidant agents and reduce the gastrointestinal absorption of toxic metals through competitive mechanisms (Abd Wahil et al. 2021; Jan et al. 2015). Pasternak and Kiełczykowska (2003) reviewed drug abuse effects on macro-and micronutrients. They report that the serum Cu in drug addicts showed an elevated status. The same results were stated for manganese (Mn) and iron (Fe) (Pasternak and Kiełczykowska, 2003). Clinical literature evidenced lower serum Zn levels in opioid-dependents (Ciubotariu et al. 2015; Sadlik et al. 2000). Non-essential metals such as lead (Pb), cadmium (Cd), and arsenic (As) do not play a role in the body's biological functions and can have detrimental health effects on exposed people (Rezaei et al. 2019).

Tramadol consumers tend to co-abuse some other substances such as opium, cigarettes, alcohol, and cannabis, and using tramadol increases the risk of using other substances (Nasiri et al. 2019). Many studies reported that cigarette smoking (Meltzer et al. 2016; Nakhaee et al. 2021a; Tawhid Hossain et al. 2018) and opium use (Alinejad et al. 2018; Azadi et al. 2021) may change the concentration of essential elements and toxic metals. But to our knowledge, there are limited studies that assessed the concentration of trace elements in tramadol dependents individuals and aggravating effects of substance abuse along with tramadol (El-Safty et al. 2018, Sabah and Al-Ameri, 2020), warranting the need for more research in this field. Therefore, this study aimed to investigate the urinary concentration of some essential and toxic elements (Ca, Cd, Cr, Mn, Cu, Zn, Co, Ni, Se, and Pb) in patients with tramadol consumption alone and in combination with cigarettes and opium use in Imam Khomeini Hospital of Kermanshah, Iran.

Materials and methods

Study population and sample collection

This case–control study adhered to Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines (Cuschieri 2019). Volunteers selected from tramadol abusers attending Imam Khomeini hospital in Kermanshah city, west of Iran, from March to November 2020. After explaining the objectives of the study the consent form was obtained. Participants were divided into two groups: case ($n = 72$) and control (62) group. The case group consisted of three sub-group: (i) pure tramadol users, (ii) those who abused tramadol along with opium, and (iii) those who consumed tramadol plus cigarette. Subjects in the control group were selected from those who accompanied the participants in the case group which was mainly one of their family members. The case and control groups were mached based on age, gender, and educational levels. Inclusion criteria of the case group included people who had a history of using tramadol with cigarettes and opium. Exclusion criteria also included groups with chronic illnesses, people with a history of other drugs co-ingestion (such as methadone or stimulants, cocaine), and people undergoing treatment. In preparing the checklist in the control group, they self-reported that they had no history of tramadol, cigarette, and opium use, while the case group included people who referred to Imam Khomeini Hospital. Opium-dependency was determined based on DSM-IV criteria and cigarette smoking were characterized based on self-reporting.

At the beginning of the study, the sample size at each group was obtained using the below formula that is used to compare two means using *t*-test:

$$n_1 = \frac{r+1}{r} \times \frac{\sigma^2 (z_{1-\beta} + z_{\alpha/2})^2}{d^2}$$

where n_1 denotes the sample size of the smaller group, r the ratio of the sample size at the larger group to the smaller group, α and β denote type I and type II errors respectively, and σ is the common standard deviation of concentration levels. The ratio d/σ is so-called Cohen "effect size" which can be defined as 0.2, 0.5, and 0.8, representing small, medium, and large differences between two groups to detect. Setting effect size to a small value, say 0.2, will require a larger sample size to detect the differences between the mean of two groups and vice versa. We defined $r = 1$ (that is equal group sizes), $\alpha = 0.05$, and $\beta = 0.2$ (that is the power of the test 80%). By assuming an effect size equal to 0.50 which represents detecting medium differences, we obtained the number of samples at each group equal to 62:

$$n_1 = \frac{1+1}{1} \times \frac{(0.84 + 1.96)^2}{(0.50)^2} = 62$$

The demographic characteristics of the subjects in both groups were recorded in a checklist including age, gender, route of administration, amount of tramadol use, duration of tramadol addiction, cigarette consumption, occupation, and education. Five-milliliter urine samples from the tramadol group who used tramadol orally or by mix with opium and cigarette were collected through referral to the Imam Khomeini Hospital in Kermanshah city, Western Iran, and the control group who had no history of tramadol, opioid, and cigarette exposure participated voluntarily in this study. After collecting urine samples, samples were capped, labeled, and kept in the refrigerator at − 20 °C until analyses. Both groups entered this study with informed consent. This study was approved by the Research and ethics committee of Birjand University of Medical Sciences (IR.KUMS.REC.1398.954).

Element analyses

In this study, urine samples were digested with a mixture of nitric acid and perchloric acid (2:1v/v) (Azadi et al. 2022). For acid digestion, 1 cc of each urine sample was transferred into 25 ml of glass test tubes. The amount of 5 cc of nitric acid (Merck, Germany) with a purity of 65% was added to each of the urine samples, and the mixture was kept at room temperature for one night until the digestion was done slowly. Then, 2.5 cc perchloric acid (72%, Merck, Germany) was added to the mixed specimens and placed in a hot water bath (Bain-Marie) for 4 h at 98 °C until complete digestion (Azadi et al. 2022). After completing the digestion, the samples were cold at ambient temperature and the samples were diluted with 25 ml deionized water. Finally, samples prepared for heavy metal readings were measured by an Agilent 7900 ICP-MS. It should be noted that all the standard solutions used for the analysis of the metal were prepared from the Merck standard at a concentration of 1000 ppm. The concentration of heavy (calcium, chromium, manganese, iron, cobalt, nickel, copper, zinc, selenium, cadmium, lead, and arsenic) in this study is in micrograms per deciliter.

Statistical evaluation

Results were presented as mean ± SD or median and quartile range for numerical variables and number (percentage) for categorical variables. To assess the differences in subject characteristics, a *t*-test or chi-squared test was used as appropriate. Normality assumption was assessed using the D'Agostino test. Spearman correlation test was used to obtain the correlation of trace elements in the two groups of tramadol and control groups. To guard against normality violation of data and also extreme outliers, robust one-way ANOVA was used to make comparisons between groups (Wilcox 2011). Moreover, the rank-based robust regression analysis was used to assess the effect of multi-factor covariates, group, age, and gender, on metal concentration levels.

Results

Participants

Of 134 participants in this study, 50 (37.31%) were female (case group, 48%; control group, 52%) and 84 participants (62.69%) were male (57.14% of cases and 42.86% of controls). The age of participants ranged from 17 to 51 years with a mean age of 28.63 ± 7.05 (cases, 28.19 ± 7.07; controls, 29.13 ± 7.06). Furthermore, about half of the participants had a high school degree, one-fourth with an academic degree, and 25% were illiterate or completed a primary school level only (Table 1). Both case and control groups were comparable in terms of their age, sex, and education levels ($p > 0.05$).

Of 72 tramadol dependents in case group, 11 subjects (15%) admitted to use tramadol and opium, 31 subjects (43%) to consume tramadol with cigarette smoking, and 30 (42%) to abuse tramadol alone without cigarette or any other forms of opiates (Table 1). Table 1 also reports the duration of tramadol use (year) as well as the number of tramadol pills used weekly by tramadol-dependent groups. The weekly average of the number of tramadol pills intake by cases was 21.36 ± 14.69 (*min* = 7, *max* = 70) with the mean of 5.07 ± 5.19 years of usage duration (median = 3.5; range: 1–30 years). Tramadol abusers who smoked cigarette had a history of 7 (± 4.79) years of addiction followed by 4.72 (± 4.56), and 3.2 (± 5.23) years in "tramadol + opium" and "pure tramadol" user groups, respectively. Kruskal–Wallis test was used to compare the median between three groups. Tramadol-dependent groups were different in terms of the duration of tramadol use and also the number of pills intake.

Table 1 Demographic characteristics of participants

	Case group ($n = 72$)	Control group ($n = 62$)	Total ($n = 134$)	*P*-value
Age (year)	28.19 ± 7.07	29.13 ± 7.06	28.63 ± 7.05	0.458
Gender				
Male	48 (66.7%)	36 (58%)	84 (37.3%)	0.397
Female	24 (33.3%)	26 (42%)	50 (62.7%)	
Education level				
Primary school	18 (25%)	16 (25.8%)	34 (25.4%)	0.361
High school	39 (54.2%)	27 (43.5%)	66 (49.2%)	
Academic degree	15 (20.8%)	19 (30.6%)	34 (25.4%)	
Tramadol-dependent group				
	Tramadol ($n = 30$)	Tramadol + opium ($n = 11$)	Tramadol + cigarette ($n = 31$)	
Number of pills (week)	24.82 ± 16.07	25.74 ± 15.16	15.57 ± 11.91	**0.004**
Duration (year)	4.72 ± 4.56	7 ± 4.79	3.2 ± 5.23	**< 0.001**

Trace element concentrations

Table 2 reports the median and quantile range of the urinary levels of trace elements at each group. Fe was the only element markedly higher among controls as compared to tramadol dependents (3.01 vs 1.52; 98.03%↑), $p < 0.001$). The concentration levels of As appeared to be same among both groups, but the levels of other elements; Ca (96.5 vs 82.5; 16.94%↑), Cd (2.05 vs 0.79; 159.49%↑), Cr (3.35 vs 4.88; 9.63%↑), Mn (8.34 vs 7.13; 16.97%↑), Cu (9.27 vs 7.59; 22.13%↑), Zn (890.36 vs 749.13; 18.85%↑), Co (1.91 vs 0.99; 92.93%↑), Ni (2.18 vs 1.25; 74.4%↑), Se (93.56 vs 80.85; 15.72%↑), and Pb (13.26 vs 5.08; 161.02%↑) were all significantly higher among tramadol dependents as compared to control group.

To investigate whether the co-abuse of tramadol with smoking cigarettes and opium affects the concentration levels of trace elements, comparisons were also made between tramadol dependent groups (cases who abused tramadol purely, tramadol in conjunction with cigarette, and joint use of tramadol and opium). Concentration levels across these groups were also compared with the control group (Table 3). Robust one-way ANOVA analysis revealed no statistical difference in concentration levels of As and Cr between four groups, but concentration levels of Ca, Cd, Mn, Fe, Cu, Zn, Co, Ni, Se, and Pb were different between groups. The only discrepancy between these finding findings and the results of Mann–Whitney *U*-test presented in Table 2 is that robust ANOVA triggered Cr as an element with non-significant levels between all groups.

Table 2 The urinary levels of trace elements (μg $L.^{-1}$) among both tramadol users and controls were presented as median (25th–75th percentile)

Element	Case ($n=72$)	Control ($n=62$)	Total ($n=134$)	*p*-value
As*	3.60 (2.42–5.42)	3.39 (4.26–4.58)	3.57 (2.31–5.15)	0.389
Ca	96.51 (83.59–107.22)	82.53 (74.52–92.73)	86.45 (76.59–102.99)	**< 0.001**
Cd	2.05 (1.22–3.07)	0.79 (0.62–1.14)	1.21 (0.79–2.07)	**0.002**
Cr	5.35 (4.63–8.25)	4.88 (3.14–7.91)	5.16 (4.36–8.10)	**< 0.001**
Mn	8.34 (6.38–9.65)	7.13 (5.51–8.38)	7.87 (5.8–9.24)	**0.004**
Fe	1.52 (1.33–1.70)	3.01 (2.71–3.34)	1.95 (1.51–2.96	**< 0.001**
Cu	9.27 (8.71–9.83)	7.63 (7.26–8.62)	8.73 (7.68–9.40)	**< 0.001**
Zn	890.36 (809.39–1042.65)	749.13 (687.84–868.06)	833.99 (711.05–976.19)	**< 0.001**
Co	1.91 (1.26–2.50)	0.99 (0.76–1.32)	1.28 (0.98–2.22)	**< 0.001**
Ni	2.18 (1.29–2.73)	1.25 (0.88–1.54)	1.52 (1.0–2.31)	**< 0.001**
Se	93.56 (90.50–103.31)	80.85 (72.89–84.03)	88.93 (79.31–96.92)	**< 0.001**
Pb	13.26 (10.92–14.90)	5.08 (4.15–6.49)	10.22 (5.29–13.42)	**< 0.001**

**As* arsenic, *Ca* calcium, *Cd* cadmium, *Cr* chromium, *Mn* manganese, *Fe* iron, *Cu* copper, *Zn* xinc, *Co* cobalt, *Ni* nickel, *Se* selenium, and *Pb* lead

Table 3 The urinary levels of trace elements (μg $L.^{-1}$) among tramadol-dependent groups (pure tramadol, tramadol with cigarette, and tramadol with opium consumers) and control group presented as median (25th–75th percentile)

Element	Tramadol ($n=30$)	Tramadol + opium ($n=11$)	Tramadol + smoking ($n=31$)	Control ($n=62$)	*p*-value
As	3.32 (2.38–4.96)	3.54 (1.33–4.87)	4.35 (3.05–6.24)	3.39 (4.26–4.58)	0.313
Ca	90.74 (83.67–108.97)	85.2 (73.88–100.47)	95.33 (84.25–105.45)	82.53 (74.52–92.73)	**0.006**
Cd	2.07 (1.23–2.91)	2.21 (1.14–3.08)	1.65 (1.24–3.18)	0.79 (0.62–1.14)	**< 0.001**
Cr	5.05 (4.54–6.93)	8.09 (4.70–8.92)	6.72 (5.05–8.17)	4.88 (3.14–7.91)	0.122
Mn	8.47 (7.21–9.76)	6.99 (5.56–7.83)	8.51 (7.05–9.78)	7.13 (5.51–8.38)	**0.006**
Fe	1.57 (1.35–1.76)	1.33 (1.19–1.48)	1.52 (1.40–1.70)	3.01 (2.71–3.34)	**< 0.001**
Cu	9.3 (8.88–9.84)	9.21 (8.91–9.65)	9.27 (8.65–9.84)	7.63 (7.26–8.62)	**< 0.001**
Zn	880.62 (726.16–1039.70)	917.38 (861.40–957.03)	914.19 (838.02–1055.30)	749.13 (687.84–868.06)	**< 0.001**
Co	1.56 (1.23–2.27)	1.75 (1.39–2.39)	2.28 (1.28–2.85)	0.99 (0.76–1.32)	**< 0.001**
Ni	2.01 (1.08–2.74)	2.19 (1.60–2.80)	2.15 (1.16–2.66)	1.25 (0.88–1.54)	**0.001**
Se	94.87 (90.62–105.66)	93.99 (91.80–101.24)	91.43 (83.07–101.35)	80.85 (72.89–84.03)	**< 0.001**
Pb	12.66 (10.94–14.66)	11.47 (10.93–18.00)	13.86 (11.05–15.06)	5.08 (4.15–6.49)	**< 0.001**

As arsenic, *Ca* calcium, *Cd* cadmium), *Cr* chromium, *Mn* manganese, *Fe* iron, *Cu* copper, *Zn* zinc, *Co* cobalt, *Ni* nickel, *Se* selenium, and *Pb* lead

Figure 1 displays the percentage increase in the 20% trimmed mean of the concentrations of trace elements in tramadol-dependent groups as compared to the control group. Increased percentage obtained was calculated as $100 \times (\overline{x}^{Tr}_{trim} - \overline{x}^{Ctrl}_{trim})/\overline{x}^{Ctrl}_{trim}$ where $\overline{x}^{Tr}_{trim}$ and $\overline{x}^{Ctrl}_{trim}$ denotes the trimmed mean of the concentrations in tramadol and control groups respectively. In Fig. 1, the *y*-axis represents trace elements sorted from the lowest increase to the highest increase in the trimmed mean of concentration levels. For instance, Fe levels decreased (negative sign) by 48% in both pure tramadol dependent and "tramadol + smoking" groups, and a 56% decrease happened with cases consuming both tramadol and opium as compared to the control group. The highlighted trace elements (Cr and As) in Fig. 1 represent no significant percentage changes with tramadol-dependent groups when compared to the control group. Pb showed the highest percentage increase among tramadol-dependent groups as compared to the control group so that the percentage increase was 141%, 154%, and 155% respectively among pure tramadol, tramadol + cigarret, and tramadol + opium consumers. Adjusted for type I error pairwise comparisons between studied groups of trace elements with significant changes found by robust one-way ANOVA (Table 3) showed that concentration levels of all elements did not change significantly between tramadol-dependent groups. In other words, significant results (*p*-values less than 5% in Table 3) were due to the differences in the trimmed mean between control and other groups.

Age, sex, and smoking effects

Table 3 suggests the significant difference between the concentrations of tramadol consumers as compared to control for all trace elements and then As and Cr. To investigate the factors that may contribute to these differences, we employed rank-based regression analysis to assess whether the concentrations of trace elements were affected by the sex, smoking habit, education, and age of participants in both general ($n = 134$) and tramadol user ($n = 72$) populations. In the general population, no contribution of sex and age effect was found by the regression model on the levels of all 12 studied elements. Smoking was found to affect the levels of Fe −0.163, $P = 0.025$), and Co ($\beta = 0.411$, $p < 0.001$) so that smoking reduced Fe levels but elevated Co concentrations. Moreover, Mn levels were the only element found to be affected by the education levels of individuals so those with an academic degree appeared to trigger lower levels of Mn compared to those with a high school level of education ($\beta = -0.156$, $P = 0.023$).

For each participant in case group, the number of tramadol pills taken per week (mean = 21.36, $SD = 14.69$) and also the duration of consumption (mean = 5.07, $SD = 5.18$) were available. Since tramadol consumption is related to

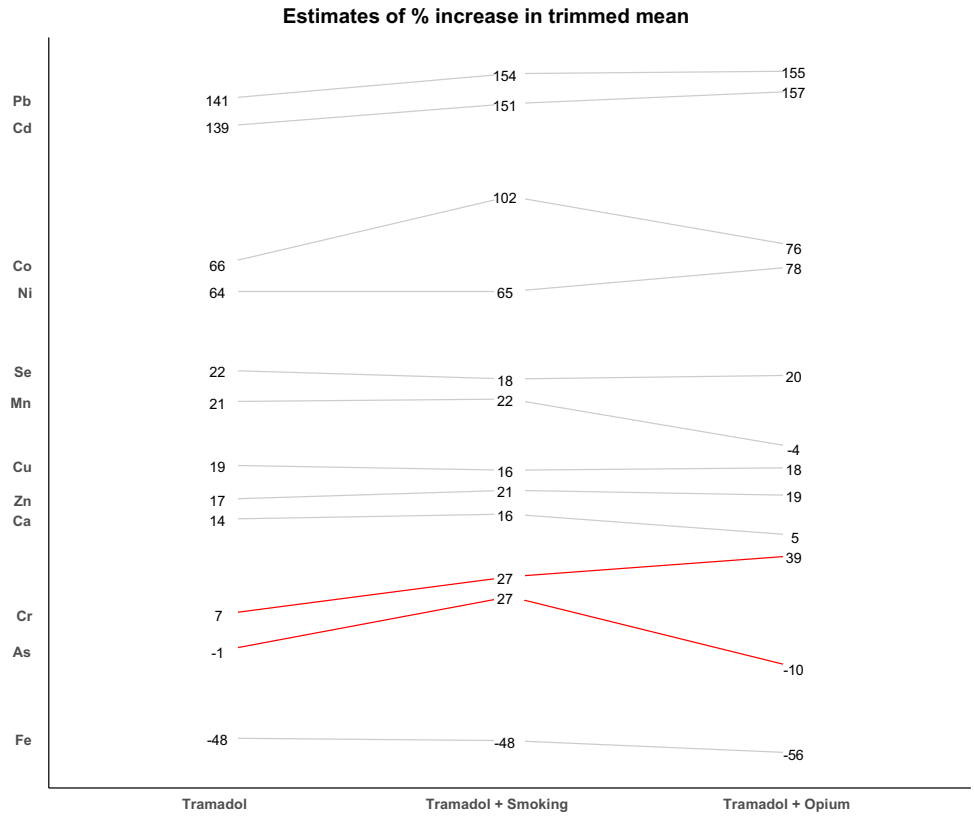

Fig. 1 Percentage increase in the 20% trimmed mean of the concentrations of trace elements in tramadol dependent groups as compared to the control group

both duration and also tramadol dosage, a composite variable was obtained by multiplying the duration of tramadol consumption and the number of pills taken per week. This variable was used to reflect the actual use of tramadol by participants in the case group (mean = 6965, *SD* = 8980.41, range: 364–46,800). Figure 2 displays the histogram bins of this composite variable which shows clearly a highly right-skewed plot. To deal with the present skewness in Fig. 2, we applied a logarithmic transformation of the values and performed a robust regression analysis using each element as the response variable and age, sex, smoking habit, education levels, and the use of tramadol volume as independent variables. Interestingly, results showed no significant effect of age, smoking habits, and tramadol usage on the levels of trace elements. Sex was only a significant factor for Ca (β = -0.125, P = 0.029) so that women had higher levels of Ca as compared to men. Education levels were found to be associated with Cr and Se concentrations so that when compared to high school levels of education, cases above high school education revealed to have higher levels of Cr (β = 0.288, P = 0.017) and cases under high school education level revealed to have higher levels of Se (β = 0.110, P = 0.002).

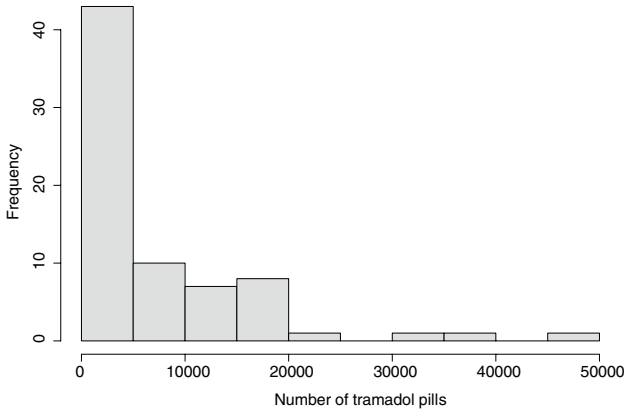

Fig. 2 The histogram plot of the number of tramadol pills claimed by the case group throughout their historical usage

Correlation analysis

Figures 3 and 4 show how trace elements are correlated for case and control groups respectively. Correlation values were obtained using Spearman correlation coefficients. The significant correlation pairs at the nominal 5% level were highlighted as either blue if positive or orange if the correlation was negative. As it can be seen clearly from both figures, the correlation between pair elements is not strong and all correlations are below 0.35 which is considered as

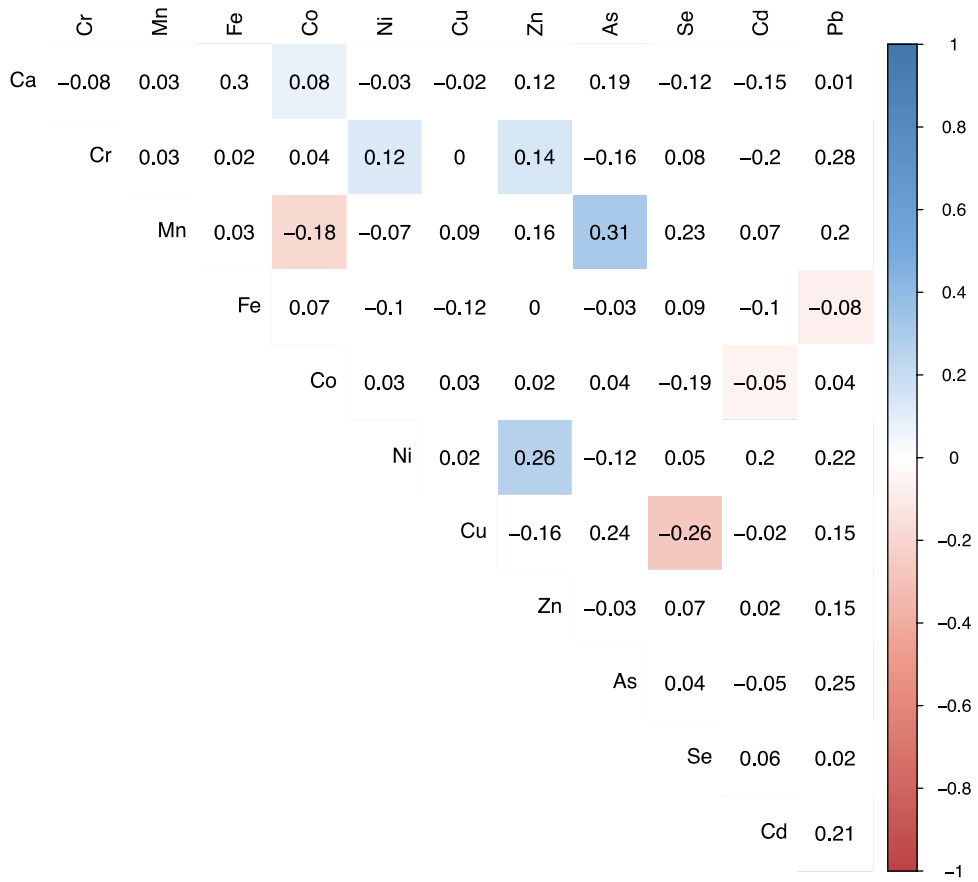

Fig. 3 Correlations between trace elements for tramadol dependents group

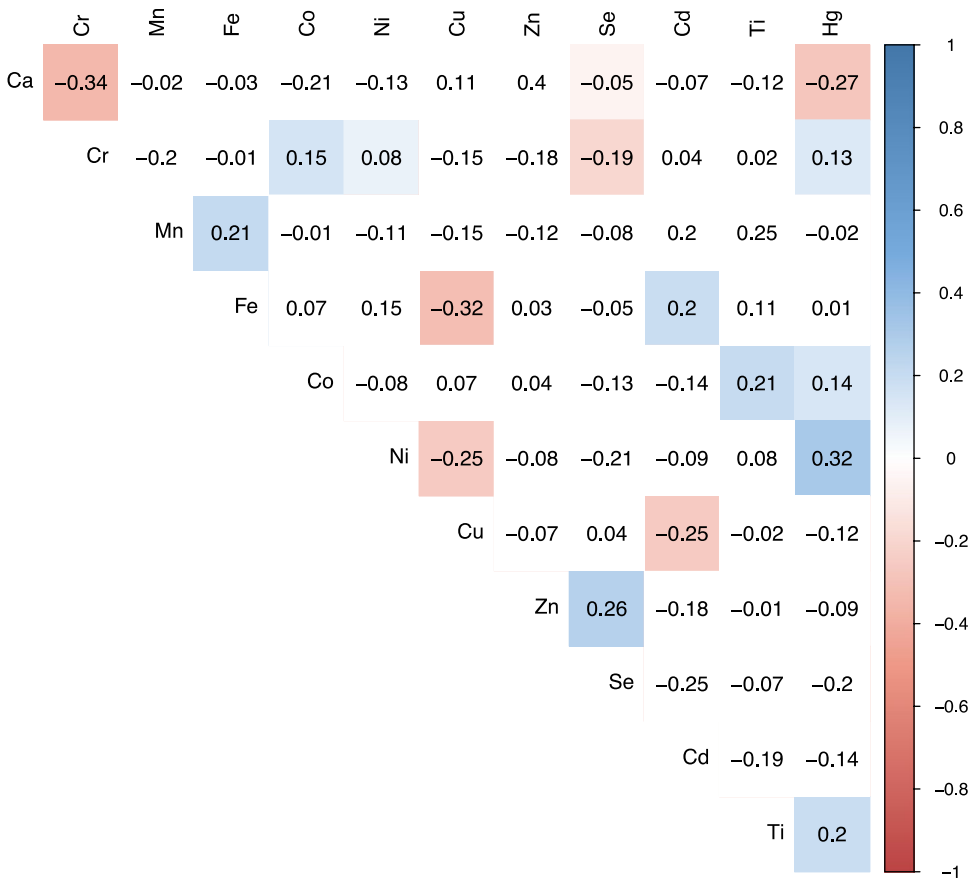

Fig. 4 Correlation between trace elements for the control group

a weak correlation. Pairwise correlation between duration of tramadol consumption (year) and trace elements concentrations (µg/L) in Table 4 shows the duration of tramadol consumption was correlated with As concentration ($r = 0.35$, $p < 0.05$) so that higher level of As accumulation is to be expected as the duration of tramadol usage increases.

Discussion

Our results showed that urinary concentrations of Ca, Zn, Cu, Co, Se, Mn, Cd, Cr, Ni, and Pb were significantly higher and Fe concentration was lower among tramadol users. To our knowledge, there are limited studies that assessed most of these elements in tramadol dependents, warranting the need for more research in this field. The essential trace elements (such as magnesium, zinc, and iron) play an important role as antioxidant agents. They also reduce the gastrointestinal absorption of toxic metals through competitive mechanisms (Abd Wahil et al. 2021; Jan et al. 2015). Calcium is considered the most abundant divalent ion (Ca^{2+}) in the body (El-Safty et al. 2018). Research on the influence of tramadol or other opioids on Ca levels revealed contradictory results. A human study reported that tramadol addiction did not induce the structural integrity of the proximal tubules but it resulted in high urinary excretion of Ca in the group of Egyptian tramadol addicts ($n = 18$), suggesting that tramadol addiction may impair nephron reabsorption function regarding urinary calcium (El-Safty et al. 2018). The effects of tramadol on serum levels of calcium mostly showed non-significant results; for example, an experimental study showed that chronic administration of tramadol (10 mg/kg for 90 days) had no significant effect on the serum calcium concentration in tramadol compared to control rats

Table 4 Pairwise correlation between duration of tramadol consumption (year) and concentrations of different trace elements (µg/L)

Duration of Tramadol consumption (year)											
Ca	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Cd	Pb
0.17	−0.06	0.20	0.03	0.01	−0.08	−0.02	0.01	**0.35***	−0.02	−0.10	0.21

*P-value < 0.05

($n = 10$ rats in each group). Also, it resulted in non-significant osteoporotic effects (Boshra 2011). Elyazji et al.(2015) demonstrated that administration of tramadol (40 mg/kg/day) increased non-significantly serum levels of calcium in rabbits (Elyazji et al. 2015). Also, in another study, no significant changes in serum Ca levels were reported in tramadol-treated animals (40 and 80 mg/kg) compared to the control (Mousavi et al. 2021). The difference in the design, participants, sample size, and endpoint may lead to controversy in the results of previous studies.

Elevated urinary calcium concentration received support from the previous reports that opioids can increase levels of growth hormone and vasopressin (Seyfried and Hester, 2012). Growth hormone excess has been reported to cause hypercalciuria, which appears to be a tubular effect. Also, vasopressin may increase urinary calcium excretion due to the reducing effect on calcium transfer in the thick ascending limb (Dirks 1988).

Zinc (Zn) is an essential element that is critical in the body's metabolic and physiological activities. In contrast to our results, some studies observed no significant difference in the urinary Zn excretion in tramadol users compared to the control group (El-Safty et al. 2018). Sadlik et al. (2000) reported low Zn serum levels in heroin dependents on admission for treatment, with a small tendency to elevate during detoxification, but not yet reached the levels in the control group (Sadlik et al. 2000). Based on the results of a review study, human and experimental literature documented lower serum Zn levels under opioid-use conditions, attributed mostly to increased urinary exertion in humans or redistribution in animals. In addition, experimental studies have shown decreased brain Zn levels and increased hepatic Zn levels in morphine-treated animals and also increased endogenous opioid activity and a possible decrease in morphine withdrawal by Zn (Ciubotariu et al. 2015). Most human studies did not consider the type of opioid, or possible effects of other drugs or co-existent diseases; also, most of them did not evidence the relationship between the period of use, the severity of addiction, and decreased Zn status. Alongside the effects of opioids on Zn status, some researchers highlighted the effects of this element on opioid pharmacodynamics. The results of a study suggest that Zn deficiency may potentiate opioid use, and they need to increase the amount of opioid consumption to achieve similar analgesia as that of Zn-normal patients (Tantillo et al. 2021). While systemic Zn administration increased tramadol analgesic effects. A study showed that Zn, Mg, and Mn administration enhanced tramadol-induced analgesia evaluated by tail-flick and hot-plate assay in Swiss male mice (Alexa et al. 2015). It is hypothesized that Zn oxidizes sulfhydryl groups of opioid receptors using a reversible redox reaction, resulting in the blockade of opioid binding to its receptor (Ciubotariu et al. 2015; Tantillo et al. 2021).

Copper (Cu) is an important element for the activity of many cellular enzymes (El-Safty et al. 2018). Contrary to our results, El-Safty et al. (2018) reported no significant difference in the urinary Cu elimination in tramadol addicted ($n = 18$) compared to the control group ($n = 19$) and tramadol addiction could not affect the integrity of nephron for Cu reabsorption (El-Safty et al. 2018). Barbosa et al. (2020) using an experimental study reflected iron metabolism changes and elevated serum Fe concentration upon exposure to different doses of tramadol (10, 25, and 50 mg/kg) (Barbosa et al. 2020). Unsaturated Fe-binding capacity and total Fe-binding capacity (TIBC) were significantly increased in heroin/opioid dependents than in controls, while hemoglobin, hematocrit, and serum Fe concentrations were significantly decreased (Guzel et al. 2018).

Co-abuse of cigarette smoking and opium with tramadol showed more elevation levels in some trace elements. Pb and Cd showed the highest percent increase in an ascending trend among pure tramadol, tramadol + cigarette, and tramadol + opium consumers, respectively. Many studies reported a higher blood lead concentration (BLC) in cigarette smokers than in non-smokers (Beshgetoor and Hambidge, 1998, Meltzer et al. 2016, Shaper et al. 1982) and it could be considered as an additional source of heavy metals. It has been revealed that smoking 20 cigarettes in a day can lead to the inhalation of 1 to 5 μg of Pb (Ashraf 2012). Also, higher Cd levels have been reported previously in cigarette smokers (Charania et al. 2014; Hecht et al. 2016).

On the other hand, Cd is considered an IARC group 1 human carcinogen with a long biological half-life (about 20–30 years), and it accumulates due to cigarette smoking (Pappas 2011). Also, previous reports, consistent with our results, documented higher concentrations of As (Bates et al. 1995, Chen et al., 2004, Lindberg et al. 2006), and Cu (Dubick and Keen, 1991), and lower Fe levels (Meltzer et al. 2016) in cigarette smokers compared to healthy controls. Some elements may increase indirectly as a part of the inflammatory response due to smoking. Based on the results of this study, opium consumption along with tramadol abuse may exacerbate the elevation level of some elements such as Pb, Cd, and Cr. It has been documented a high tendency of the opium poppy to accumulate toxic metals, particularly Cd and Pb (Lachman et al. 2006). According to previous studies, the presence of lead in opium samples caused higher BLC in opium users (Aghababaei et al. 2018, Aghaee-Afshar et al. 2008, Amirabadizadeh et al. 2020, Domeneh et al. 2014, Ghaemi et al. 2017, Ivan and Jozef, 2011, Khatibi-Moghadam et al. 2016, Mohsen Masoodi et al. 2006). The effects of this toxic metal on the pharmacodynamics of opioids have also been highlighted (Kupnicka et al. 2020). It can disrupt the neural pathways associated with addiction development. It can affect dopamine metabolism and the expression of dopamine receptors (Kupnicka et al. 2020).

The existence of Cd in various parts of the opium poppy has been reported, previously. (Aghababaei et al. 2018, Ivan and Jozef, 2011, Knapek et al., 2009, 2011, Lachman et al. 2006). Also, its antagonistic effects on μ-opioid receptors and inhibitory effects on the release of dopamine and glutamate receptors have been shown (Kupnicka et al. 2020; Lafuente et al. 2000; Smith et al. 2002). Some experimental studies reported a reduced response to morphine due to Cd exposure (Smith et al. 2002).

Our results showed no significant effect of age and tramadol dose on the levels of trace elements. But gender was only a significant factor for urinary Ca levels. The sex differences in Ca levels of tramadol users encountered in our study could be attributed to opioid-induced endocrinopathy. One of the potential mechanisms for opioid-induced sex hormone deficiency is the hypothalamic-pituitary–gonadal axis suppression (Colameco and Coren, 2009). Daily opioid consumption for chronic pain has been reported to cause reduces in adrenal androgen production in women dose-dependently (Daniell 2008). It has been shown that estrogen deficiency leads to malabsorption of calcium which may be associated with decreased intestinal vitamin D receptors (Gallagher et al. 2001). Boshra (2011) observed a lower serum calcium level in the rats treated with morphine or fentanyl. They attributed this observation to a reduction of albumin in serum due to estrogen deficiency and an increase in urinary calcium levels (Boshra 2011). To our knowledge, the effects of age, dose of tramadol, and educational levels on trace element levels in the tramadol users have not been well studied. It determines the need for future researches. The key message of this study to be conveyed is that co-abuse of cigarette smoking and opium with tramadol may exacerbate some trace elements levels. More comprehensive research is needed to elucidate these results.

Limitation

It should be noted that due to the nature of concentrations of trace elements, data are usually right-skewed and also contain extreme values. This makes the use of parametric approaches which benefit from higher power less efficient. One limitation was to use non-parametric or robust approaches instead which require larger sample sizes. Another challenge in statistical analysis was the unbalanced nature of studied groups which affects the efficiency of statistical methods. For instance, the subjects in the "tramadol + opium" group were only 11 cases which further reduced by 20% when analyzed by robust ANOVA. Lower sample sizes are known to reduce the power of analysis which in turn leads to non-significant results. Moreover, in the current study, toxic and essential elements were evaluated only in urine samples, which may not fully elucidate the complex pathological mechanism caused by tramadol in the body. Another limitation was the lack of control over participants' diets, feeding habits, and environmental factors. Therefore, it is not evident if trace element changes are an effect of tramadol or an associated condition in opioid users. But as the subjects in the control group were selected from those who was mainly one of family members of case group, the case and control groups have similarities for their diatray habits and environmental conditions. Assessing how tramadol addiction affects trace elements with considering the nutritional status and environmental factors using a large-scale study suggests a future field of study. In the current study, opium-dependency was determined based on DSM-IV criteria and cigarette smoking was characterized based on self-reporting. Unfortunately, assessing biomarker such as urinary levels of cotinine, morphine, and tramadol to characterize the cigarette, opium, and tramadol abuse was not possible for researchers.

Conclusion

In summary, we observed that urinary concentrations of Ca, Zn, Cu, Co, Se, Mn, Cd, Cr, Ni, and Pb were significantly higher and Fe concentration was lower among tramadol users compared to controls. Results showed no significant effect of age, smoking habit, and amount of tramadol usage on the levels of trace elements. Based on the results of this study, opium consumption along with tramadol abuse may exacerbate the elevation level of some elements such as Pb, Cd, and Cr. These data are useful in that they extend the information available about tramadol; clinicians should use tramadol in chronic pain considering the trace element concentration and the effects of toxic metal on the pharmacodynamics of opioids and progress of dependence.

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Author contributions VF, SN, NA, MK, TJ, and BM contributed to the design of the study, the interpretation of the results, and the drafting of the manuscript. MK and BM conducted the collection of the data. NA and BM conducted the statistical analyses. All authors have read and approved the final version of the manuscript.

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Availability of data and materials The datasets used and analyzed during the current research are available from the corresponding author on request.

Declarations

Ethics approval and consent to participate This study was conducted by the World Medical Association Declaration of Helsinki. This study was approved by the Research and Ethics Committee of Kermanshah University of Medical Sciences (IR.KUMS.REC.1398.954).

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

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