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RESEARCH ARTICLE

Determination of toxic elemental levels in whey milk of different cattle and human using an innovative digestion method: risk assessment for children < 6.0 months to 5 years

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Abstract

In present study, the toxic elements, arsenic (As), cadmium (Cd), and lead (Pb), were determined in whey milk samples obtained from various cattle (cow, goat, buffalo, sheep, camel) and human subjects of different areas of Sindh, Pakistan, based on consuming drinking water (exposed area) and surface water (control/non-exposed area). The whey milk was separated from casein by lowering the pH, and heating in an ultrasonic bath at 60 °C for 5 min and centrifuged. The whey milk samples were treated with deep eutectic solvent, prepared from choline chloride–oxalic acid (ChCl–Ox) at different mole ratio. Effects of different parameters on digestion efficiency of whey milk samples, including time and temperature of electric hot plate, mole ratio, and volumes of deep eutectic solvent were examined. The total levels of all selected toxic elements were also detected in whole milk samples of all exposed and nonexposed cattle and human, after acid digestion method. The validity of the proposed method was established by a conventional acid digestion method of selected whey milk samples and spiked certified standards in replicate real whey milk samples. The resulted elements obtained after proposed and conventional heating system were determined by inductively coupled plasma–optical emission spectrometry. The % of all three toxic elements found in whey milk samples were 24 to 50% of their total content in milk samples of different cattle and human. The As, Cd, and Pb contents in cattle and human milk consumed contaminated groundwater was significantly higher (2- to 3-fold) than those values observed for milk samples of cattle, who receive drinking water from fresh canal water ($p < 0.01$). Estimating the daily intake, hazard quotient and carcinogenic risk for <6 month to 5 years old children, based on the concentrations of toxic elements in milk samples of different cattle and human.

Keywords Deep eutectic solvent; Cattle · Human milk · Toxic elements · Risk assessment

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Highlights

- Toxic elements were determined in whey milk of cattle and human subjects drinking contaminated water.
- An innovative digestion method was used to analyze toxic elements in whey milk samples of cattle.
- The deep eutectic solvent, prepared from choline chloride–oxalic acid used as extraction solvent.
- Toxic elements in milk samples of cattle and human correlate to their drinking water.
- Toxic elements in whey milk of cattle were 24 to 50% of their total levels in milk.
- The HQ value of toxic elements was > 1, creating adverse impact on health of children.

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Introduction

In recent years, due to the deterioration of environment, the contents of harmful elements, such as arsenic (As), cadmium (Cd), and lead (Pb) are possibly increasing in the food commodities including milk, which are harmful to the health of people of all age group, especially infants and children (Ping et al. 2012). The food chain is an important source of toxic elements when the plants grown on polluted agricultural land with irrigation water. However, the transfer of toxic elements from contaminated soil/water to plants and grass, causing direct impact on grazing ruminants, especially in cattle (Alonso et al. 2002, 2003; Malhat et al. 2012). The milk and milk products are important natural food, and they contain more than 20 minor and trace elements. It was reported that the levels of trace and toxic elements varied

according to the types of cattle, their feed, and drinking water (Al-Wabel 2008). Whey milk is the liquid substance left after milk clots or when casein is removed during cheese production (Brandelli et al. 2015). There is a potential role of whey milk as a supplement with considerable amount of macro and micronutrients (Hoac et al. 2007).

As the milk is essential feed of children < 5 years, the contaminated milk with toxic elements induced different physiological disorders including mental retardation in initial life (Rahimi 2013). The intake of contaminated milk may also be a causative factor for arsenical disorders in human especially in children and elders (Sigrist et al. 2010), its chronic exposure, through drinking water and food, has been associated with lower IQ, poor and diminished intellectual and cognitive functioning in the long run (Tyler and Allan 2014). Researchers have extensively examined that the contamination of breast milk with As, Cd, and Pb related with sociodemographic status and dietary habits of nursing mothers (Bassila et al. 2018). The Cd accumulates in different body parts, especially kidneys and liver throughout the life, so it makes the most dangerous characteristic on human health (Satarug 2018). The exposure of Pb for longer periods creates harmful effect on the human body and its high level in the blood is only partially reversible and then later decline (Tajkarimi et al. 2008).

In addition to food, the contaminated milk with toxic elements can also have harmful impact on humans particularly infants, children, and old people (Cai et al. 2009; Alonso et al. 2003). During lactation, the toxic elements might be transported from maternal plasma to mammary glands. These toxins can seriously damage infant's nervous system once it reaches the breast milk (Bellinger 2008; Dorea 2004). So, the international organizations pay particular attention to determine the toxic elemental levels in milk of humans and cattle.

For detecting the trace and toxic elements, most common techniques are flame atomic absorption spectrometry (Sikiric et al. 2003), graphite furnace atomic absorption spectrometry (Kazi et al. 2009), and inductively coupled plasma–optical emission spectrometry (Barin et al. 2012). Direct determination of elements in milk may be difficult due to matrix interferences and their low concentrations. So, these techniques require extraction/digestion of milk samples to remove the complex matrices. For preparation of the samples, different heating devices such as conventional heating (electric hot plate), ultrasonic bath and microwave oven were used (Manutsewee et al. 2007; Neves et al. 2009). However, the disadvantageous of acids or oxidizing agents assisted digestion methods, produces highly carcinogenic nitrous and other acidic vapors after destruction of the organic matrices. To avoid these problems, the mild environmentally friendly reagents are beneficial for digestion/extraction of elemental contents in foods (Neves et al. 2009).

The trace and toxic elements in food matrices are extracted by different methods and techniques (Sacmacı et al. 2011; Sacmacı and Kartal 2011; Sacmacı and Sacmacı 2021). The ionic liquids (ILs), which are capable as green solvents due to their low pressure, high boiling points, and have selectivity for different applications, such as chemical or enzymatic reactions (Martín-Calero et al. 2011). However, many reports indicated that the hazardous toxicity and poor biodegradability of some ILs used for the enrichment and preconcentration of elements from food matrices (Petkovic et al. 2011). A new class of solvents deep eutectic solvents (DESs), derived from mixing of two components are overcome the problems related to waste generation and disposal of acidic digestion mixture (Abbott et al. 2006; Abbott et al. 2006; Yilmaz and Soylak 2015). However, the DESs have similar physicochemical properties as ILs (Abbott et al. 2006; Habibi et al. 2013). The DESs are normally prepared by mixing ammonium salts (such as choline chloride), hydrogen bond donors (HBD), urea, and carboxylic acids (Abbott et al. 2006; Zhang et al. 2012). It was reported in a study that variety of metal oxides are soluble in ChCl-based eutectic solvents in the range of 0.3–18300 µg/mL at 50 °C (Kazi et al. 2019; Abbott et al. 2006).

Beside the conventional heating system, ultrasound energy has attained great interest as an efficient heating and shaking/dispersion of solution as well as cost-effectiveness (Arain et al. 2017 2017; Neves et al. 2009). Ultrasound energy is capable to increase mass transfer between two media by creating a cavitation effect in solution, due to physical phenomena such as micro-streaming, acoustic (or shock) waves, and micro-jets as they propagate through the solution (Asfaram et al. 2016).

Pakistan produces about 33 billion liters of milk per year, making as the 4th largest milk producer country in the world. There are a large number of buffaloes, cows, sheep, camels, and goats in Pakistan approximately 30.8, 34.3, 27.8, 1.0, and 59.9 million, respectively (FAOSTAT 2010). There is limited information present in research article about the concentrations of toxic elements in milk samples of cattle (camels, cows, goat, sheep, and buffaloes) and childbearing women in Pakistan belong to different areas. Exposure of toxic elements to newborn and children ≤ 2 years via consuming contaminated cattle and human milk are of special interest.

The objective of this study was to evaluate the effects of As, Cd, and Pb in livestock drinking water, an important source of exposure on milk producing cattle. In the present study, the livestock drinking water (ground and surface water) and milk samples of sheep (*Ovis aries*), goats (*Capra hircus*), cows (*Bos taurus*), buffaloes (*Bubalus bubalis*), and camels (*Camelus*) of different farms/flocks of Tharparkar, Pakistan, were analyzed for As, Cd, and Pb. The human milk samples were obtained from both areas where women

consuming groundwater and fresh water (control). In addition, it is reported for the first time the application of a deep eutectic solvent (ChCl and oxalic acid) for the disintegration of matrices of whey milk to solubilize the toxic elements (As, Cd, and Pb), prior to their determination by inductively coupled plasma optical emission spectrometer. The different variables, including the composition and volume of DES (ChCl: Ox), temperature, time of dissolution and treatment with dilute acid and water. For comparative purpose, the whey milk samples were subjected to conventional acid digestion method. Simultaneously, the composite milk samples of each cattle and human were analyzed for total toxic elemental contents by acid digestion method. The estimated daily intake and risk assessment (carcinogenic risk factor and hazardous quotients) of toxic elements via consuming milk of cattle and human for < 6-month- to 5-year-old children were calculated.

Materials and methods

Chemical and reagents

All chemicals were of analytical reagent grade and solutions prepared with ultrapure water. A certified stock standard solutions of As, Cd, and Pb (1000 µg/mL) was acquired from Fluka Kamika, Switzerland. Before analysis, the working standard solutions were obtained by appropriate dilution of the stock standard solutions. Choline chloride (ChCl) has purity > 98%, was obtained from Sigma- Aldrich. The oxalic acid (Ox) with high purity was purchased from Merck Company (Darmstadt, Germany). Trichloro-acetic acid (TCA), obtained from Merck, was used for sedimentation of whey proteins.

Instrumentation

The measurement of toxic elements was conducted by inductively coupled plasma emission spectrometry (ICP-OES), Bremen, Germany. The phase separation was assisted with a centrifuge ROWKA Laboratoryjna typeWE-1, nr-6933 (Mechanika Phecyzyjna, Poland). A programmable ultrasonic water bath, model no. SC-121TH (Sonicor, Deep Park, NY, USA), was used for incubation with temperature ranging from 0 to 80 °C at intensification frequency of 35 kHz.

Sampling of milk and water

Milk samples were collected from different cattle ($n = 50$ of each) belongs to two areas based on livestock drinking water, obtained from freshwater canal (rural areas of Hyderabad city) where many farms and flocks are present, as nonexposed area. The groundwater samples had been collected from 3 farms of cow, buffaloes, and 3 flocks (sheep, goat, and camel) from Tharparkar Pakistan ($n =$ 10–20 of each) during 2020 termed as exposed samples. The sampling of groundwater was carried out manually from dug well with the depth > 40 ft. The water sampling points were noticed with the help of GPS (global positioning system). The women of both nonexposed and exposed area were selected for sampling of breast milk after 2 to 3 months of delivery date ($n = 50$). All the samples were treated on same day in laboratory and made ten composite samples of each cattle and human milk obtained from both areas and kept at − 4 °C till further treatment. The ground and surface water samples were analyzed for As, Cd, and Pb by reported literature (Brahman et al. 2014; 2016; Kazi et al. 2020).

Preparation of whey milk

Triplicate of each composite milk samples (50 mL) of different cattle and human of both exposed and nonexposed areas were taken in flasks (100 mL in capacity). Then lowering the pH of whole milk to 4.0 by acetic acid (0.2 mol/L); after that, the flasks were placed in an ultrasonic bath at 60 °C for 10 min. Then kept the contents of flasks at room temperature and centrifuged at 3500 rpm for 20 min in order to isolate the precipitate, contains fat and casein micelles. The separated whey milk samples were further treated with 1% of TCA and NaCl (1 mL of each) for sedimentation of whey protein. The contents of tubes were vortexed for 2 min and again centrifuged for 10 min at 3500 rpm, then separated the upper clear phase by a pasture pipette and used in a developed digestion method.

Preparation of DES

The immense numbers of deep eutectic solvent (DES) are synthesized by different easily available chemical compounds such as salts and hydrogen bond donors (Abbott et al. 2004). In the present study, the DES was synthesized from choline chloride (ChCl) and oxalic acid at different compositions (1:1, 1:2, and 1:3 mole ratios) and applied for digestion of whey milk samples. The synthesis of DES from ChCl and oxalic acid at different composition was taken in round-bottom flask and heated on electric hot plat at control temperature (50 °C), with constant stirring. The temperature of the flask contents was constantly checked with a thermometer till a consistent liquid was obtained.

Digestion of whey milk by DES

For digestion/extraction of whey milk samples, taken two sets of replicates, three samples of each whey milk samples (1.0 mL) of all cattle and human belong to exposed

and nonexposed areas in tubes (50 mL in capacity). Simultaneously, spiked the certified standards of all elements in real whey milk samples. In the next step, added 1.0 to 3.0 mL of DES (ChCl–Ox) with three compositions, then placed the tubes in ultrasonic bath for 10 to 30 min. After that, the contents of one set of flasks were treated with 5 mL of 0.2 mol/L of HNO_3, whereas 5.0 mL of deionized water was added to the contents of the tubes (2nd set). The contents of all tubes were stirred for 5 min and subjected to centrifugation for 5 min at 3000 rpm. The supernatant solution was carefully separated and diluted up to 10 mL in volumetric flasks with deionized water.

For comparative purpose, triplicate of each whey milk (2.0 mL) was treated with HNO_3 and H_2O_2 (2:1 ratio) and heated on hot plate at 80 °C, till clear solution was obtained. Then, contents of flasks were cooled at room temperature and diluted with 0.1 mol/L of HNO_3 up to 10 mL. A blank solution was also conducted without any samples/standards. The all-prepared solutions were subjected to ICP-OES for analyzing the levels of As, Cd, and Pb in whey milk and compared with those values obtained by proposed DES-assisted extraction/digestion method.

Determination of total toxic elements in milk samples

Taken duplicate whole milk samples of all cattle and human (2.0 mL) in flasks (50 mL in capacity), treated with 2 mL of HNO_3 + 0.5 mL sulfuric acid (H_2SO_4), and 1 mL of H_2O_2. Then kept all flasks at room temperature and shaken for 10 min manually then placed on electric hot plate at 80 °C for 2 h. After cooling at room temperature, added ultrapure water to made volume 25 mL in volumetric flasks (Table 1).

Statistical analysis

Data processing and statistical analysis were conducted by using computer program Excel 2003 (Microsoft Office ®), XLState (Addinsoft, NY, USA), Minitab 13.2(Minitab Inc., State College, PA) software packages. The resulted data of triplicate samples of each composite samples (n = 10 of each) were expressed as mean ± standard deviation. The results were analyzed statistically by one-way ANOVA. The Student's test was used to assess the significant difference of spiked toxic elements in real whey milk samples and experimentally found values. The significance of differences between mean values of studied elements was estimated by the Tukey's range test at $p > 0.05$ and $p < 0.01$.

Table 1 Validation of the proposed method for determination of toxic elements in certified reference material of water using ICP-OES

SRM 1643e (μg L^{-1})	Certified values	Measured values	%RSD	% Recovery[b]	Paired t-test,[c] $t_{Experimental}$
As	60.4 ± 0.72	59.2 ± 2.2[a]	3.62	98.0	0.473
Cd	6.57 ± 0.073	6.45 ± 0.25	3.81	98.3	0.355
Pb	19.6 ± 0.21	19.3 ± 1.2	4.32	98.3	0.328
Standard addition of As, Cd and Pb in a real whey milk samples of cow					
Elements	Added conc. (μg/L)	Mean ± SD[a] (μg/L)		%RSD	% Recovery
As	0.00	3.74 ± 0.12		----	---
	5.00	8.61 ± 0.41		4.6	98.4
	10.0	13.5 ± 0.52		3.7	98.2
Cd	0.00	1.62 ± 0.21		---	---
	2.00	3.67 ± 0.14		2.7	98.6
	5.00	6.52 ± 0.27		4.1	98.2
Pb	0.00	4.27 ± 0.21		---	---
	5.00	9.12 ± 0.34		3.2	98.4
	10.0	14.2 ± 0.45		2.8	98.1

[a]Mean value ± at 95% confidence interval ($p = 0.05$)

[b] Percent recovery = $\text{Experimental values} / \text{certified value} \times 100$

[c]Paired t-test between certified values/ vs found values, degree of freedom (n=5)

$t_{Critical}$ at 95 % confidence limit = 2.57

[d] Values in parenthesis indicate %RSD

[b]% recovery = $\text{Found values} - \text{values with 0 addtion} / added \times 100$

Results and discussion

Impacts of different variable on proposed digestion method

Effects of ChCl–oxalic acid ratio and volume

As the ratio of two compounds to prepare DES is very important for its applicability. In the present study, the ammonium salt of choline chloride (ChCl) was mixed with organic acid (oxalic acid) at diverse mole ratio, as indicated in section 2.3, to synthesize three compositions of ChCl–oxalic acid (1:1, 1:2, and 1:3). It was observed that the best solubilization of selected toxic elements was observed at 1:2 ratio of ChCl/oxalic acid, in whey milk samples of different cattle and human (Fig. 1). However, at 1:3 ratio of ChCl/oxalic acid, no significant difference was observed ($p < 0.05$). For further experimental work, 1:2 ratio of ChCl/oxalic acid was selected. It was immensely investigated by Abbott et al. (2004, 2005, 2006) that the DES prepared from organic/carboxylic acid provides considerable competences for the solubilization of metal compound as compared to other kind DES, which may be due to the protons of the carboxylic acids, act as oxygen acceptors from the complexes of the metal and metalloids, form the chlorometalate species.

The volume of deep eutectic solvent was used in the range of 0.5 to 2.0 mL for the digestion of all three elements in whey milk samples. The optimum recoveries of As, Cd and Pb was achieved at 1.0 mL. Therefore, 1.0 mL of deep eutectic solvent was selected as optimum volume for further study.

Effect of temperature and heating time

The temperature and digestion/extraction time (whey milk and DES) of ultrasonic bath were studied for the optimum recovery of all three selected toxic elements at the range of 70 to 90 °C for 10 to 30 min, respectively. It can be shown in Fig. 2 that enhancing the temperature improves the extraction efficiency of selected toxic elements; however, the optimum extraction effectiveness was achieved from 80 to 90 °C. Consequently, 80 °C was selected as optimal temperature for further experimental work. It was observed that at 60 to 70 °C, the As and Cd recoveries were > 96%, whereas 5 to 10% lower extraction recovery was observed for Pb. However, the %recoveries of all three elements were ≥ 98% at 80 °C. Figure 3 indicates that optimum extraction efficiency for As and Cd was obtained after heating for 15 min; however, optimal extraction efficiency of Pb requires 20 min. Therefore, subsequent experimental work was carried out for 20-min heating time.

The proposed digestion method was compared through conventional wet acid digestion using concentrated acid and oxidizing mixture and heating duration of > 60 min at temperature of 100 °C on electric hot plate, to obtain clear solution. The resulted data indicated that the recoveries of selected elements were 1 to 2% higher than those values obtained after DES-based digestion/extraction, whereas difference was not significant ($p > 0.05$). The main beneficent is that the heating of proposed digestion method can be carried out outside the hood, due to the low vapor pressure of the DES, as well as avoid the use of concentrated acids/oxidizing agents. The severe disadvantage of acid digestion is that the strong acid or oxidizing reagents causes interference in the measurement step. Due to consumption of strong acid (HNO_3), development of highly carcinogenic nitrous or other

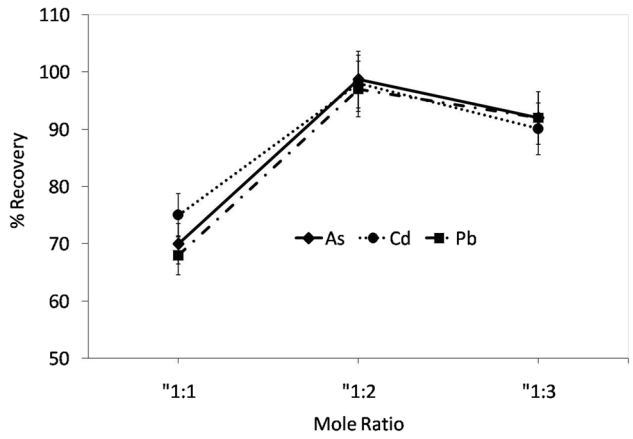

Fig. 1 Effect of the composition of DES (ChCl:Ox) on the recovery of As, Cd, and Pb ($n = 3$). Parameters: volume of DES (2.0 mL); temperature (90 °C); time (25 min); 5 mL of 0.2 mol/L of HNO_3

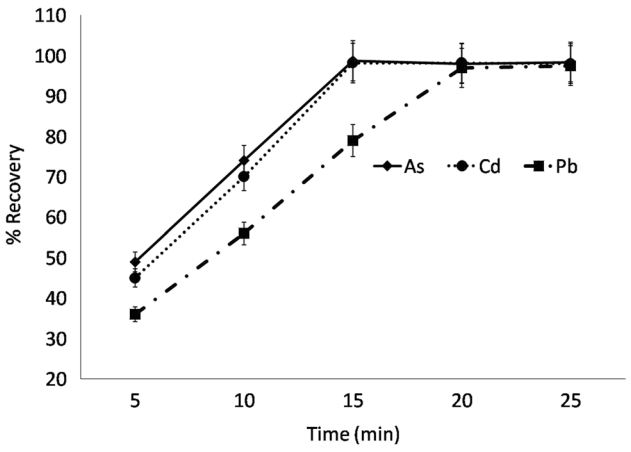

Fig. 2 Effect of the time of sample dissolution on the recovery of As, Cd, and Pb ($n = 3$). Conditions: volume of ChCl:Ox (1:2), 2.0 mL; temperature,90◦C; 5 mL of 0.2 mol/L of HNO_3

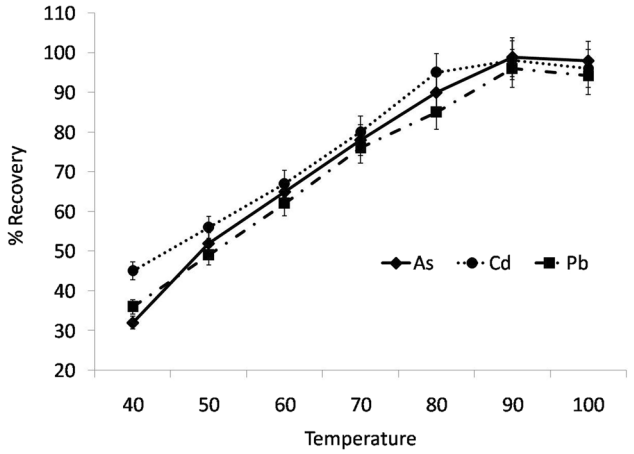

Fig. 3 Effect of the temperature of sample dissolution on the recovery of As, Cd, and Pb ($n = 3$). Conditions: volume of ChCl:Ox (1:2), 2.0 mL; time, 25 min; 5 mL of 0.2 mol/L of HNO_3

acidic fumes after organic matrix destruction is another disadvantage of these methods.

Effect of acid addition on recoveries of toxic elements

At the end of dissolution/extraction of toxic elements in whey milk samples of cattle and human, with best possible condition of all variables such as 1.0 mL of DES at composition of 1:2 ratio with the optimized settings of temperature and heating time, addition of 5 mL of dilute HNO_3 (0.2 mol/L) to the contents of the flasks and for comparative purpose in other set of flasks, 5 mL of ultrapure water was added. There was no significant difference in extraction efficiencies of As and Cd (> 98%) was observed without acid addition ($p > 0.05$), whereas the recovery of Pb was significantly lower (93%, $p < 0.01$). However, for further experimental work diluted acid was added after the end of the proposed digestion method (Habibi et al. 2013).

Toxic elements in livestock drinking water and milk samples of cattle and human

The concentration of As in livestock drinking water of two selected areas and milk samples of all cattle and human as mean values with standard deviation are shown in Tables 2 and 3. The As level in the groundwater and surface canals water termed as nonexposed area (NEA) was found in the range of 125–260 µg/L and 10.2–16.4 µg/L, respectively (Table 2). The maximum permissible levels of As in livestock drinking water as recommended by national legislation as 50 µg/L (US National Research Council 2001). The level of As in milk samples of cow and buffaloes of different farms belongs to EXA was found in the range of 23.2–32.5 and 15.6–20.2 µg/L, respectively. The concentration of As in milk samples of cow and buffaloes belongs to farm nearby the Hyderabad city (NEA) consuming fresh water of canal were ranged as 10.4–14.8 and 7.8–10.2 µg/L, respectively. The As levels in the milk samples of buffaloes was about 25 to 30% lower than cow milk (Table 3). The levels of As in whey milk samples of exposed cow and buffaloes were found in the range of 8.1–12.4 and 4.67–7.27 µg/L corresponding to 30–46% and 27–42% of its total contents in milk samples of both cattle, respectively. It was observed that whey milk of buffaloes contained significantly lower values of As than cow belongs to same area ($p < 0.01$). The As concentration in the camel's milk samples of exposed area (Tharparkar) ranged between 22.4 and 27.6 µg/L, whereas milk samples of camel obtained from control area were found in the range of 9.25–12.8 µg/L, which is ≤ than permissible limit for drinking water. The concentrations of As in whey milk samples of camel were found in the range of 8.28–11.0 µg/L, corresponding to 36–48% of its total contents. The total As levels in the milk samples of sheep and goat collected from EXA were ranged as 23.5 to 36.4 and 24.0 to 40.2 µg/L (Table 3). The levels of As in whey milk samples of sheep and goat were observed as 13.5–14.7 and 13.4–17.3 µg/L, corresponding to 45–49% and 42–54% of the total As contents in their milk samples, respectively. The As in whey milk of goat and sheep are not significantly different ($p < 0.05$). The concentration of total As in human milk samples of nonexposed and exposed women were ranged as 2.63–3.24 and 15.6–18.4 µg/L, respectively. The As level in whey milk of women belongs to NEX and EXA were observed in the range of 0.735–0.894 and 4.31–5.04 µg/L, respectively, corresponding to 28 to 33% of its total contents in milk. The As levels was significantly lower in milk samples of human belongs to both EXA and NEX as compared to cattle ($p > 0.01$).

The levels of As was found to be significantly higher in studied goat milk samples than literature reported values, 10.0–24 µg/L (Antunovic et al. 2005) and 0.9–28 µg/L (Rosas et al. 1999). It was inferred that As content in milk samples varied from place to place depending on its concentration in drinking water, which is also used for irrigation purpose, and may accumulate in grass and other plants (Brahman et al. 2014). Immense investigation has been carried out about contamination of milk with toxic elements

Table 2 Determination of toxic elements in ground and surface water

Toxic elements	Groundwater	Surface water	WHO permissible limit
	µg/L		
As	188 ± 48	13.4 ± 2.2	10.0
Cd	20.4 ± 3.8	5.6 ± 0.86	3.0
Pb	97.5 ± 3.6	18.3 ± 1.6	10.0

Table 3 Total toxic elements in milk and their whey samples (μg/L) of cattle and human belongs to exposed and nonexposed area (n = 30 of each)

Cattle	Arsenic		Cadmium		Lead	
	NEA[a]	EXA[b]	NEA	EXA	NEA	EXA
Camel	11.5 ± 0.85 (4.07 ± 0.78)[c]	23.2 ± 3.6 (9.66 ± 1.1)	4.24 ± 0.2 (1.50 ± 0.32)	10.3 ± 0.34 (3.95 ± 0.42)	13.5 ± 0.88 (4.41 ± 0.86)	27.5 ± 2.5 (9.39 ± 1.5)
Cow	12.5 ± 1.2 (3.74 ± 0.68)	27.0 ± 2.6 (10.3 ± 1.2)	3.85 ± 0.1 (1.62 ± 0.24)	9.78 ± 0.23 (4.25 ± 0.53)	11.8 ± 0.65 (4.27 ± 0.76)	25.5 ± 2.1 (8.68 ± 1.1)
Sheep	14.4 ± 0.92 (5.90 ± 0.82)	30.4 ± 2.5 (14.1 ± 1.8)	3.75 ± 0.24 (1.23 ± 0.28)	12.6 ± 0.45 (5.05 ± 0.67)	8.45 ± 0.74 (2.86 ± 0.47)	32.2 ± 3.1 (10.0 ± 1.8)
Goat	12.4 ± 0.82 (4.84 ± 0.76)	32.5 ± 1.2 (15.4 ± 1.6)	3.56 ± 0.11 (1.37 ± 0.15)	16.3 ± 0.54 (6.54 ± 0.87)	13.2 ± 0.83 (4.80 ± 0.84)	36.7 ± 3.5 (10.7 ± 1.8)
Buffaloes	8.54 ± 0.74 (2.72 ± 0.13)	17.3 ± 2.9 (5.96 ± 0.85)	3.78 ± 0.34 (1.25 ± 0.13)	10.8 ± 0.75 (3.55 ± 0.67)	12.3 ± 0.88 (4.33 ± 0.523)	28.8 ± 2.6 (9.24 ± 1.6)
Human	2.63 ± 0.15 (0.736 ± 0.04)	14.5 ± 1.6 (4.06 ± 0.73)	1.58 ± 0.042 (0.49 ± 0.03)	6.45 ± 0.51 (1.72 ± 0.32)	6.85 ± 0.43 (2.12 ± 0.38)	21.2 ± 1.3 (6.27 ± 0.74)

[a]Nonexposed area

[b]Exposed area

[c]Values in parenthesis ( ) toxic elements in whey milk

in different regions, indicated the comparable results of As, whereas lower levels of As in milk samples of cow was observed than reported study (Licata et al. 2004). Whereas lower values of As in milk samples of cow were observed as reported in other study (Simsek et al. 2000).

The total Cd levels in livestock drinking water of two selected areas and milk samples of all cattle and human are shown in Tables 2 and 3. The Cd level in the groundwater of exposed areas/villages, where different farms and flocks involve different cattle, was found in the range of 13.5–25.6 μg/L. The levels of Cd in surface water samples (canal) were found in the ranges of 2.32–4.65 μg/L (Table 2). The national legislation establishes as maximum tolerable limit for Cd in water designated by WHO (3 μg/L). The levels of Cd in milk samples of cow and buffaloes of different farms belongs to EXA were found in the range of 9.25–10.5 and 9.64–10.9 μg/L, respectively. In the case of Cd, no any significant difference was observed among milk samples of cow and buffaloes ($p > 0.05$). The concentration of Cd in whey milk samples of cows and buffaloes of EXA found in the range of 3.04–3.42 μg/kg equivalent to 28 to 35% of its total contents in milk samples. It was observed that the concentration of Cd in whey milk of cow was about 12% higher than buffalos' milk of EXA ($p > 0.05$). The Cd levels in cow and buffalos belonging to farms near the Hyderabad city consuming freshwater of canal were found in the range of 3.56–3.25 and 3.45–4.32 μg/kg, respectively. The levels of Cd in whey milk samples of cow and buffaloes of NEX were found in the range of 0.95–1.34 μg/L and 0.78–1.09 μg/L corresponding to 28 to 34% and 27 to 32% of its total contents in milk samples, respectively. The Cd concentration in the camel's milk samples of EXA (Tharparkar) were ranged in between 9.2 and 11.6 μg/L. Whereas, milk samples of camel obtained from the flock present in NEX area found in the range of 3.87–4.8 μg/L, which were three folds lower than those values obtained in milk samples of exposed camel ($p > 0.01$). The levels of Cd in whey milk samples of camel belonging to EXA and NEX were found in the range of 2.8–3.2 and 1.27–1.73 μg/L corresponding to 28 to 34% and 30 to 41% of its total contents in camel milk, respectively. The concentration of total Cd in human milk samples of nonexposed and exposed women were found in the range of 1.46–1.72 and 7.2–8.4 μg/L, respectively. The concentration of Cd in whey of human milk samples of EXA and NEX were ranged as 2.42–3.15 and 0.55–0.73 μg/L, respectively, corresponding to 31 to 38% of total Cd contents in their milk samples. It was observed that available Cd in whey milk of EXA was found to be ≤ 3.0 μg/L, within the permissible limit of it in drinking water. The concentration of Cd in milk samples of nonexposed cattle was not significantly different from each other ($p > 0.05$), whereas the human milk contains 2-fold lower level of Cd as related to all cattle ($p < 0.01$).

It was indicated in literature that the concentrations of Cd determined in milk in different countries were as follows: below than detection level of 0.006 μg/kg in different farms from South Africa (Ataro et al. 2008), 1–6 μg/kg in southern Poland (Krelowska-Kulas et al. 1999), 0.01–22.80 μg/kg in Calabria, Italy (Licata et al. 2004), and 0.47 μg/kg and 0.40 μg/kg in Spain (Martino et al. 2001; Sola-Larranaga and Navarro-Blasco 2009). In contrast, levels of Cd found in the milk sample of different cattle were lower in comparison with milk from unpolluted

Table 4 Estimated daily intake, hazardous quotient, and cancer risk values of As, Cd, and Pb, due to consumption of milk of different cattle and human for infant to 5-year-old children

	Nonexposed area			Exposed area		
	Age group 1	Age group 2	Age group 3	Age group 1	Age group 2	Age group 3
Age (years)	0–1	1–2	2–5	0–1	1–2	2–5
Human milk	Arsenic					
EDI[a] (µg/kg/day)	0.39	0.18	-	2.92	1.31	-
HQ[b]	1.95	0.87	-	14.5	6.56	-
CR[c]	5.85×10^{-5}	2.63×10^{-5}	-	4.38×10^{-4}	1.97×10^{-4}	-
Cow milk						
EDI	1.39	0.63	0.39	3.00	1.35	0.84
HQ	6.94	3.12	1.95	15.0	6.75	4.23
CR	2.0×10^{-4}	9.3×10^{-5}	5.8×10^{-5}	4.5×10^{-3}	2.1×10^{-4}	1.26×10^{-4}
Goat milk						
EDI (µg/kg/day)	1.71	0.772	0.483	3.56	1.60	1.00
HQ	8.56	3.85	2.41	17.78	8.00	5.00
CR	2.6×10^{-3}	1.2×10^{-3}	7.0×10^{-4}	5.3×10^{-3}	2.4×10^{-3}	1.5×10^{-3}
Human milk	Cadmium					
EDI (µg/kg/day)	0.262	0.124	-	1.08	0.482	-
HQ	0.1006	0.045	-	0.408	0.183	-
CR	1×10^{-4}	4.50×10^{-5}	-	4.09×10^{-4}	1.84×10^{-4}	-
Cow milk						
EDI (µg/kg/day)	0.432	0.192	0.123	1.09	0.493	0.314
HQ	0.163	0.073	0.045	0.412	0.185	0.116
CR	1.62×10^{-4}	7.31×10^{-5}	4.5×10^{-5}	4.12×10^{-4}	1.85×10^{-4}	1.16×10^{-4}
Goat milk						
EDI (µg/kg/day)	0.40	0.178	0.11	1.82	0.817	0.51
HQ	0.1503	0.0676	0.0423	0.6899	0.3105	0.1940
CR	1.5×10^{-4}	6.7×10^{-5}	4.23×10^{-5}	6.8×10^{-4}	3.10×10^{-4}	1.94×10^{-4}
Human milk	Lead					
EDI (µg/kg/day)	1.14	0.51	-	3.54	1.59	-
HQ	0.0024	0.001	-	0.0075	0.0033	-
CR	9.70×10^{-6}	4.37×10^{-6}	-	3.01×10^{-5}	1.35×10^{-5}	-

Table 4 (continued)

	Nonexposed area			Exposed area		
	Age group 1	Age group 2	Age group 3	Age group 1	Age group 2	Age group 3
Age (years)	0–1	1–2	2–5	0–1	1–2	2–5
Cow milk						
EDI (µg/kg/day)	1.32	0.59	0.37	2.83	1.27	0.80
HQ	0.0028	0.0013	0.0008	0.0060	0.0027	0.0017
CR	1.11×10^{-5}	5.03×10^{-6}	3.14×10^{-6}	2.4×10^{-5}	1.08×10^{-5}	6.7×10^{-6}
Goat milk						
EDI (µg/kg/day)	1.47	0.662	0.412	4.08	1.84	1.15
HQ	0.0031	0.0014	0.0009	0.0087	0.0039	0.0024
CR	1.2×10^{-5}	6.4×10^{-6}	4.2×10^{-6}	3.5×10^{-5}	1.6×10^{-6}	1.0×10^{-5}

[a]Estimated daily intake

[b]Hazardous quotients

[c]Carcinogenic risk

areas (0.033 mg/kg) and regions around plants and smelters (0.057–0.265 mg/kg) in India (Patra et al. 2008).

The Pb level in the groundwater of EXA was ranged as 75.5 to 120 µg/L. The levels of Pb in fresh surface water samples (canal) were found in the ranges of 15.5–20.2 µg/L. The national legislation establishes as maximum tolerable limit for Pb in water designated by WHO (10 µg/L).The total contents of Pb in milk samples of nonexposed camel, cow, sheep, goat, and buffaloes were found in the range of 12.5–14.2, 10.8–12.7, 7.6–9.2, 12.6–14.3, and 11.7–13.8, respectively, whereas milk samples of cattle belong to exposed area contains Pb concentrations as 26.7–29.2, 24.3–26.8, 28.6–34.5, 35.2–37.4, and 27.5–29.2 µg/L, respectively. It was observed that Pb values in milk samples of exposed cattle were 2- to 3-fold higher than those values in milk obtained from cattle consumed fresh canal water ($p < 0.01$). The Pb contents in whey milk of camel, cow, sheep, goat, and buffaloes belonging to NEX were found in range of 3.2–3.8, 2.7–3.1, 2.5–3.2, 3.1–3.44, and 2.85–3.22 µg/L, respectively, corresponding to 23 to 28% of its total contents in milk samples. Whereas, in whey milk of exposed cattle, camel, cow, sheep, goat, buffaloes contains Pb ranged as 5.5–7.8, 6.7–8.4, 7.2–8.1, 6.0–7.3, 6.45–7.82 µg/L, respectively, were corresponding to 24–28% of its total contents in their milk samples. The Pb concentrations in human milk of nonexposed and exposed areas were found in the range of 6.85–7.65 and 21.2–24.4 µg/L, respectively. The Pb concentration in whey milk of human corresponding to 22–28% of its total contents in milk samples of women of both areas (Table 3). The Pb levels in human milk was significantly lower as compared to cattle of both areas ($p < 0.01$).

It was reported by Bilandzic et al. (2011) that the cow milk samples of different farms in Croatia contains lead concentrations 36.2 to 58.7 µg/L; these values are considerably higher than the present results ($p > 0.01$). It was reported that the Pb concentration in the milk of cows from organic farms was much lower than present work, ranged as 4.1 to 6.2 µg/L (Gabryszuk et al. 2010). It was indicated in another study the comparable results of Pb in cow milk (Simsek et al. 2000). The significantly higher concentration of Cd and Pb in cow milk samples as compared to present results (Pavlovic et al. 2004). Interaction between toxic heavy metals (Pb and Cd) and major nutritional and trace elements was also found in humans, most frequently in blood and serum (Barany et al. 2002) and in the milk of nursing mothers (Stawarz et al. 2007).

The Spearman's correlation was calculated among toxic elements in water and milk samples of cattle and human. The correlation among groundwater used as livestock drinking water in exposed area and milk samples of larger cattle (cow and camel) were found to be significantly higher ($r =$ 0.845 to 0.88, $p = 0.01$), than those values obtained from smaller cattle ($r = 0.465$ to 0.583). The reason may be due to difference in water consumption rate, the large quantity of drinking water consumed by camel and cow as compare with the goats and sheep including lactating women. So, the elevated values of correlation coefficient indicated that the content of toxic elements in the milk sample of different cattle depends on their amount in drinking water.

Health risk to children via consuming milk of different cattle and human

The nutritional status of children aged < 6 months to 5 years via consumption of cattle and human milk of both exposed and nonexposed areas are evaluated. However, in the present study, other sources of toxic elements such as food and drinking water not involved. The information about consumption of milk obtained from various cattle and their amount were collected from selected families of both areas. The estimated daily intake of toxic elements via consuming cattle and human milk for children of various age group (< 0.6 months to 5 years) was calculated based on the equation reported elsewhere (Copat et al. 2012; Shaikh et al. 2019). The EDI was expressed in μg/kg bw/day. The average concentrations of As, Cd, and Pb in milk samples of different cattle, and volume consumed by children such as 0.75 L/day for < 2 years, with normal body weight of selected children group (Kent 2006). Based on the equation cited elsewhere, estimating the daily intake (EDI), exposure dose, hazard quotient (HQ), and cancer risk (CR) for children (Copat et al. 2012; Brahman et al. 2016; Shaikh et al. 2019).The calculated EDI for As, Cd, and Pb compared with the tolerable daily intake (TDI) derived from the provisional tolerable daily intake (DTWI), reference values given by FAO/WHO Expert Committee on Food Additives as 2.1, 1.0, and 3.57 μg/kg bw/day, respectively (Stankovic et al. 2011). The US EPA cancer risk considered the minimum or acceptable for regulatory purposes is within the range (1×10^{-6} to 1×10^{-4}) (Sultana et al. 2017, EPA 2011).

The EDI value of As via consuming human milk by children of age group < 0.6 months to 2 years of NEA and EXA were found as (0.39 and 0.18) and (2.92 and 1.31) μg/kg/day, respectively (Table 4). It was observed that the EDI of As for children of age group 1 to 2 years of NEA was 4- to 7-fold lower than those values observed for EXA ($p > 0.001$). The HQ for As via consuming human milk by the children of age group < 6 months and 2 years of NEA were found many folds lower (0.87–1.95) than those values observed for EXA (6.56–14.5). The CR in children of both areas was within limit (10^{-5}–10^{-4}). The EDI value for As via consuming cow milk by the children of age group 1, 2, 3–5 years of NEA were found to be about 2-fold lower than those values observed for children belongs to EXA ($p < 0.01$). The HQ value for all age groups from exposed area were > 1 than the acceptable limit (Table 4). The CR values for children of EXA (three age groups) show limit of CR (10^{-4}), but < 1 year have higher CR (10^{-3}) than elder group. However, they all are in a risk to developing various types of cancer in the future, but currently selected children do not show any signs of arsenicosis. The EDI, HQ, and CR values of As for children of < 0.6 months to 2 and 3 to 5 years belonging to EXA was > than those values, observed for all children group resided in nonexposed area. The high exposure of As through diet creates adverse impact on pregnancy outcomes and causes the death of fetus and harmful impact on the health of children < 2 years (Quansah et al. 2015). The exposure of As in early childhood increases the rate of mortality in later age might be due to multiple cancers, lung disease, heart attacks, and skin diseases (Farzan et al. 2013).

The EDI value for Cd via human milk consumption in the children of age groups 1 and 2 of EXA were found to be 4-fold higher than NEA shown in Table 4. The HQ for Cd via human milk consumption in the children of < 6 months to 2 years of EXA was found to be ≤ 1. The CR values for both age groups of EXA were found to be ≥ 10^{-4}, which shows serious health concerns to the children in future. The EDI values for the Cd via drinking cow's milk by the children of age group < 0.6 months to 5 years belonging to EXA were about 5-fold higher than those EDI values observed for children of all age groups residents of nonexposed area. The HQ of exposed and nonexposed children of all age groups was found to be > 1. The cancer risk factor for exposed and nonexposed children was found to be 10^{-3}–10^{-4} and 10^{-4}–10^{-5}. The EDI of Cd via consuming goat milk at selected dose was found to be 3-fold higher in exposed children of all three age groups than nonexposed children. The HQ value of children of all age group belonging to both areas was > 1, indicating that from early age group, high risk was occurred due to consuming goat milk. The CR value for Cd by consuming goat milk was found to be higher in children belongs to exposed area than nonexposed age-matched groups. The effect of selected toxic elements As, Cd, and Pb, with provisional tolerable weekly intake levels (PTWI) reported by Food and Nutrition Board (2001), 0.015, 0.007, and 0.025 mg/kg, respectively. The levels of these toxic elements were found to be lower in consumers of cattle and human are milk, belongs to NEA (in some cases) and EXA. The HQ value > 1 specifies the possibility of adverse effects on the health of living beings and proposes the necessity of further investigation and probable remedial action. However, HQ < 1 represents no probable adverse health effects from exposure of toxic elements at current consumption rate (EPA 2010).

The high hazardous properties of Cd are that it gathers during whole lifetime. The Cd most probably accumulates in the kidney and liver and has an extensive half-life in biological system of human, i.e., 17–30 years. The absorption of Cd increases in early childhood and with iron deficiency, enhance the number of carriers shared by toxic elements in the duodenum. The high exposure of Cd in children of early age creates three times more adverse impacts, likely to have learning disabilities (ATSDR 2012). Kippler et al. (2009) established a significant association between Cd concentration in erythrocytes and breast milk, whereas breast milk to plasma ratio is

approximately 3:4. So, Cd can be transported from plasma to breast milk without any barrier.

The levels of Pb were lower in milk samples of different cattle and human in NEA. Young children are particularly vulnerable to lead poisoning because they absorb 4–5 times as much ingested lead as adults from a given source. Exposure of Pb can have serious consequences for the health of children (Baranowska et al. 2005; Braun et al. 2008). In particular, Pb can affect children's brain development, resulting in reduced intelligence quotient (IQ) behavioral changes such as reduced attention span and increased antisocial behavior and reduced educational attainment (Braun et al. 2006). The optimal limits of Pb for milk and its products were consider as 0.05 ppm, which is the total limit in the European diet (European diet provides maximum potential for weekly intake of lead through food) would be 4.63 µg of Pb/kg body weight. The adverse impact of high exposure/intake of Pb causes adverse impacts on every living being including children in early age acts as a neurotoxin causes irreversible health effects on nervous system (Eshpari et al. 2014). The higher biological availability of Pb is prohibited and impaired the functions of cell membrane might be due to the disturbance of ion transport across it (Marinova et al. 2009).

Milk is the fundamental food for the early life period (< 1 years) and would be sustained for later period > 2 years. The most acceptable source for this age group is breast feeding, and this is greatly encouraged; however, in certain conditions, a specific diet might be required in some critical aspect (absence of mother feed), artificial feeding via cattle milk especially goat and cow milk. The neonates are more vulnerable to adverse impacts of toxic elements, which might be due to substantial disparities in their metabolism, underdeveloped excretory system (kidney), and their higher energy requirements via food such as milk of human/cattle (cow or goat) for early age groups (Haskell et al. 2006).

The exposure of toxicants to newborn via human and cattle milk are higher, which might be due to their gastric pH ranged 6–8, and the availability of toxic elements are more at this pH. The toxic effects of elements are partly due to direct inhibition of enzymatic system and also to the indirect alteration of the essential elemental ion-equilibrium. The milk whey has a significant part in relations to the bioavailability of toxic elements, because it comprises free elements (unbound) and, also, binding with the serum albumin proteins and citrate, which can be assimilated by the organism including infants, and children < 2.0 years. It was also mentioned in the literature that elevated absorption of Cd and Pb causes the interfering with essential micronutrient metabolism, especially in neonates (Al-Saleh et al. 2011).

The significant sources of toxic elements in milk and food commodities are water, particularly in those area where geological or industrial pollution are dominate (Mishra and Sharma 2011). Thus, the examination of livestock drinking water is the significant feature to evaluate the exposure of them on milk of different cattle. Another factor must be accepted when calculating exposure of toxic elements for infants because in early age of life their growth and development are very rapidly and require maximum amount of energy, so the consumption of food is much higher as relative to their body weight than adults and elder children. Presently, the infants and children especially < 5 years belonging to EXA have ill health and causality rate is high, not only due to intake of toxic elements via consuming milk of cattle and human but also due to contaminated water intake. However, the other factors such as poverty, low nutrition, and lack of Medicare facility may have synergistic effect. Our assumption is also reliable with other study (Rastogi et al. 2004). In selected age groups (1–5 years), children have no symptoms of arsenicosis; however, their parents have some evidence of rough skin and pigmentation on the palms of the hands, and soles of the feet.

Conclusion

This was the first regional study in developing country, where impact of contaminated groundwater with toxic elements and freshwater canal on milk of different cattle and humans was carried out. The simple and environmentally friendly procedure was developed to obtain the toxic elements in whey milk of cattle and human by short analysis time, lack of concentrated acids, oxidizing agents, and use of safe and inexpensive deep eutectic solvent for routine analysis. Significantly higher levels of As, Cd, and Pb was observed in milk samples of cattle and human consumed groundwater than those, who drink fresh canal water ($p < 0.01$). The levels of toxic elements in whey milk were corresponding to different % of them in whole milk. The estimated intake for all three selected toxic elements on daily basis by infants and children (age ranged < 6 months to 5 years) belonging to nonexposed areas was found to be lower value than the limit set by international guidelines, than those values observed for children belongs to exposed area. The EDI and HQ for children, drinking milk of different cattle and human, consuming groundwater of Tharparkar might be unsafe for children < 1 to 2 years. This demonstrates that particular attention should be paid to higher toxic elements in livestock and human drinking water should be controlled in future studies to confirm the absence of possible toxicological risks to exposed neonate/children. The high mortality rate of infants was occurring in Tharparkar, which might be due to poor immunity beside the malnutrition. It was further recommended to quantify the As, Cd and Pb in initial food of neonates especially in human, cow, and goat milk.

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Data availability Not applicable.

Declarations

Ethical approval Not applicable.

Consent to participate Not applicable.

Consent for publication All the authors are willing to publish this article in ESPR.

Conflict of interest The authors declare no competing interests.

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