ORIGINAL ARTICLE

LEAD IN FOOD AND ITS CORRELATION WITH BLOOD LEAD LEVELS AMONG PREGNANT WOMEN OF SINDH, PAKISTAN

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Background: With increasing control of lead (Pb) in gasoline, food has emerged as an important secondary pathway for Pb exposure globally. This study assessed Pb levels in food duplicates and blood. Furthermore, it assessed the correlation of Pb in food duplicates and blood Pb levels with common food items taken by pregnant women of Sindh, Pakistan. **Methods:** A cross-sectional study was conducted from August 2014 to November 2015, in urban (Karachi) and rural (Gambat, Khairpur) area of Sindh-Pakistan. A total of 103 venous blood samples (Karachi = 63 and Gambat = 40) of pregnant women were measured for blood Pb levels at the time of delivery. One month post-delivery, food frequency questionnaire (FFQ) was administered and three-day food duplicates (same number of cooked portions of food eaten by women) were collected. Food duplicates were analysed for Pb levels. Multivariable linear regression was conducted to identify the frequency of food items which contribute to blood and food Pb levels of pregnant women, separately. **Results:** With 90% confidence interval, *chapati* (local flat bread) (β=0.20, p<0.001), boiled rice (β=0.35, p<0.001), cooked root vegetables (β= 0.16, p=0.03), fried savory items (β=0.15, p=0.03), sweet snacks (β=0.13, p=0.08) were positively associated with elevated mother blood Pb levels. While cream biscuits (β =-0.14, p=0.04), *lassi* (blend of yogurt, water and spices) (β= -0.31, p<0.01), market milk desserts (β=-0.22, p<0.001), fish (β= -0.16, p=0.02), soft drinks (β= -0.19, p=0.01) and *supari/gutka* (betel-nut) (β=-0.13, p= 0.06) were negatively associated with mother Pb levels. Tetra-pak market juices (β= 0.30, p<0.001) and cooked root vegetables (β=0.19, p=0.05) were positively associated with mother food Pb levels, at 90% CI. **Conclusion:** Bread, boiled rice, fried savoury items, sweet snacks and cooked root vegetables were contributing to blood Pb levels of pregnant women in Pakistan. These food items may be contaminated with Pb during processing, packaging and storage. Inverse relation of *Supari/gutka* and soft drinks with blood Pb levels may be due its overall reduction in absorption capacity of the gut for nutrients. Objective individual food item analysis for Pb is warranted for further intervention.

Keywords: Mother blood Pb levels; Mother food Pb levels; Food frequency; Food duplicates; Pakistan

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INTRODUCTION

Lead (Pb) is a heavy toxic metal affecting several organs including brain, liver, kidney, blood and bones, particularly among children.[1] Pb exposure causes 853 000 deaths, accrued to 9.3% global burden of intellectual disability, 6.6% of strokes and 4% ischemic heart diseases.[2]

Newborns are exposed to Pb levels from mother's blood during pregnancy.[3,4] The Center for Disease Control and Prevention (CDC) adopted 5 µg/dL as the upper reference range value for children blood Pb as an advisory level for environmental and educational intervention[5], however consensus is building that there is no safe limit for blood Pb levels. Evidence suggest that Pb also affects the health, even at level below 5 µg/dL.[6]

Mining, smelting, leaded paints, leaded gasoline and batteries for motor vehicles are primary sources of Pb.[2] Lead has been controlled in primary sources media like petrol and paints in most of the developed countries and now secondary sources like soil, water and food have emerged as important pathways. Pakistan has also implemented Pb limits on petrol since 2001.[7]

Food has now emerged as an important pathway for increasing the blood lead levels.[2] Food can be contaminated through Pb during processing, packaging and storage.[6] Higher Pb levels have been found in plants grown around mines and smelters. Food can also be contaminated when transported from rural to urban areas as once Pb is released into environment, it persists.[6] Leaching of Pb into food from lead-glazed ceramic or pottery dinnerware may also be a source of lead exposure.[6] United States food and drug administration (FDA) has set 0.5 µg/dL as acceptable Pb level for food products intended for consumption by infants and children.[5]

Lead is used in manufacturing of cosmetics like '*surma/kajal*' (khol), which are common eye cosmetics used in South Asia and Middle East.[2] Use of *surma/kajal* in women and children has been reported to increase their blood Pb levels.[8] Pica during pregnancy has also been reported to be a risk factor in increasing pregnant women's blood Pb level and in turn of the newborn.[9,10] In Pakistan *multani matti*, a form of clay, is most commonly eaten by pregnant women experiencing pica.[11]

Limited assessments for Pb exposure through food has been conducted in Pakistan. A study in a fishing community of Karachi found 3.90 µg/g of lead in cooked food.[12] The study had limited number of participants and cooked food samples were collected only at one point in time from the households.[12] Another study from five sites of Karachi found Pb in the range of 1.25–3.90 µg/g in different food items collected from household however, the study had not described the method of food samples collection.[13] Furthermore, these studies were conducted in urban squatter settlements of Karachi more than a decade ago.[12,13] A more recently published linked case study from Karachi has shown that the food was the most important source of Pb intake among pregnant women, with *in vitro* bio accessibility of lead from food ranged between 29–83% (mean = 62.37%).[14] The contribution of Pb from food was more as compared to other sources like water, house dust, respirable dust and soil.[14]

With the above background, we conducted a study in which three days food duplicates, food records and food frequency questionnaires were used to assess the association between food and Pb levels in blood and food. This comprehensive study was conducted in urban as well as rural districts of Sindh- Pakistan to determine the association between foods as a pathway for affecting blood Pb levels by taking actual three days food duplicates of pregnant women.

MATERIAL AND METHODS

This cross-sectional study was conducted during August 2014 to November 2015 at one urban and one rural site of Sindh-Pakistan. The urban site was Karachi, which is the largest megacity of Pakistan and the rural was Gambat Taluka (an administrative unit) of Khairpur district.

Pregnant women were recruited from hospital setting (Qatar Hospital, Orangi town) of Karachi and Taluka Hospital, private clinics and through contacting lady health visitors from Gambat through non-probability sampling technique. From Karachi, 116 pregnant women agreed to participate in the study. Of them, 63 completed their questionnaires and dietary information. From Gambat 40 pregnant women completed their questionnaire, samples and dietary information. Pregnant women in gestational age 30 weeks and above, low risk pregnancy, at least one young child 1–3 years old livings with the mother, and resident of area for at least last four years were recruited in study. Pregnant women with pre-eclampsia and eclampsia, severe anaemia (Hb <7.0 g/dl), current twin pregnancy, previous still birth/ intra-uterine death, elderly grand multipara and pregnancy associated with general disease like diabetes and hypertension, were excluded. Written informed consent was taken from study participants.

Besides socio-demographic information, participants were also inquired regarding their husband's occupations. Participant's dietary information was collected through three-day food records, food frequency questionnaire (FFQ) and actual three days food duplicates (i.e., collection of all food items and fluids taken in amount consumed). This dietary information was collected during postnatal period.

Food Frequency Questionnaire (FFQ): For reporting frequency of the intake of different food items eaten in the last one month, a modified structured food frequency questionnaire (FFQ) having 62 food items was administered after one month of delivery.[15] Responses were recorded on Likert scale from less frequent intake to more frequent intake. Participants were shown dummies for estimating the accurate amount of food consumed by them. FFQ was analysed to determine the association between foods items and food Pb levels and blood Pb levels.

Food Diary: Simultaneously, the participants were also asked to fill a 24-hour diary for breakfast, lunch, snacks and dinner for all three days. They filled the food diaries with help of interviewer.

Collection and analysis of food samples: After one month of live born delivery, women were followed at their homes for food sample collection. Participants were asked to provide three days food duplicates (two weekdays and one weekend) to capture maximum variety of food eaten by them. They were given incentives in the form of cash for providing duplicates of food. Food items were collected separately for each day in steel boxes cleaned with deionized water. Liquids were collected in lead free plastic bottles. Records were made of all collected food items. Biochemical analysis of three days food duplicates was done to determine food Pb levels. These food Pb levels were used to determine the association between the food items and blood Pb levels.

The whole sample of three-days food duplicates were processed by food processor (Magimix Compact 3200XL; Magimix UK Ltd., Surrey, UK) to make homogenized mixture by adding drinking water and other drinks collected from the same household. In case of further liquid requirement for making a paste, measured amount of deionized water was used. A total amount of 150 ml of paste for each participant was shipped to Japan in lead free tubes for determining the food Pb levels. Detail of sample analysis is explained elsewhere.[14]

Collection and analysis of blood samples: Pregnant women's venous blood samples were collected through aseptic measure at the time of delivery. These samples were collected in Pb free tubes and were shipped to Japan for laboratory analysis. They were digested with 2 mL of nitric acid Ultrapur-100 (Kanto Chemical Co., Inc., Tokyo, Japan) in a microwave digestion system TOPwave (Analytik Jena Japan Co., Ltd., Kanagawa, Japan), according to the instruction manual, and were analysed by inductive coupled plasma–mass spectrometry (ICP-MS).[14]

The study was given approval by the Ethics Review Committee of Aga Khan University and the Institutional Review Board of Jichi Medical University, Japan.

Statistical Analysis: Frequencies and percentages were calculated for socio-demographic variables. Variable of socioeconomic status was computed by Principal Component Analysis with household assets variables, residence, mother's education and husband's education. Related to FFQ and food duplicates, food items consumed by less than 10% of study participants were removed for analysis. To see association between individual food item and mother food lead level (MFLL) and mother blood lead levels (MBLL), univariate linear regression analysis was done. Those food items with p-value less than or equal to 0.25 were considered for multivariate linear regression at 90% confidence Interval. For linear regression, MBLL and MFLL was log transformed. In multivariate linear regression, different models were made by removing insignificant variables based on R^2 change and p-value. SPSS version 19 was used for analysis.

RESULTS

The mean age of urban and rural participants was 25 and 32 years, respectively. About 64% participants were less than 29 years old. Only 34% of participants and their husbands had education level of matriculation. From urban area 41%, 33% and 6% participants and from rural area 10%, 20% and 70% participants were belonging to upper, middle and low socioeconomic status. Piped water and biomass fuel was used by 58% and 38% of the study participants, respectively. About 21% of husbands had led related occupation. None of the women had Pb risk occupation, 96% of women were housewives while rest were either office workers or working as maids. About 59% and 45% of study participants reported to use *surma* and *kajal (kohl)*. Around 37% of study participants experiencing pica consumed *multani matti* (clay) during their current pregnancy (Table-1).

In univariate linear regression analysis of MBLL with food items from FFQ, one unit intake of *chapati* (flat wheat bread) leads to log of -0.20 change in MBLL (CI=-0.09, -0.01, *p*-value=0.04). It means that with increase in number of *chapati*, Pb level in blood showing decrease. The intake of *chapati*, rusk, plain cake, cream biscuits, fried rice, low fat milk, yogurt/*raita*, *lassi (buttermilk)*, market milk desserts, vegetables cooked with and without potato, mutton, fish, and soft drinks were negatively associated with log of MBLL (Table-2). In multivariate analysis, one unit intake of *chapati* leads to log of 0.20 change in MBLL at 90% CI with *p*-value <0.001. It means that with increase in number of chapati, Pb level in blood showing increase when adjusted with residence in multivariate analysis. Eleven food items were statistically significant at 90% CI in final model of multivariate linear regression where cream biscuits, *lassi*, market milk desserts, fish, *supari/gutka* and soft drinks were negatively associated with log of MBLL. Bread slice, boiled rice, cooked root vegetables, fried savory items and sweet snacks were positively associated with log of MBLL (Table-2). However, when adjusted for residence, intake of bread slice, cream biscuits, *lassi*, fried savory items, sweet snacks and *supari/gutka* were insignificant (Table-2).

In univariate linear regression food items from FFQ with mother food lead levels (MFLL) of three days food duplicates; *naan (wheat bread made with yeast)*, bread slice, rusk, plain cake, plain biscuits, *khitchri* (dish of rice and lentil), fried rice, *biryani/pulao* (dish of rice and meat/chicken), whole milk, low fat milk, yogurt/*raita*, *lassi*, market milk desserts, beef, mutton, chicken, fish, organ meat, *chole/lobia* (chickpeas/beans), fried savoury items, saltish savoury items, *supati/gutka* and soft drinks were negatively associated with log of MFLL in univariate analysis. (Table-3). In multivariate analysis of food items and MFLL, seven food items were statistically significant from which intake of *lassi*, market milk desserts, beef, mutton and organ meat were negatively associated while intake of tetra-pack market juices and cooked root vegetables were positively associated with log of MFLL (Table-3). When adjusted with residence, *lassi* was insignificant. In univariate linear regression analysis of mother three days food duplicates and MBLL; *chapati*, rusk, plain biscuits, fried rice, fresh fruits, green leafy vegetables, potatoes, chicken, fish, boiled egg and tea were negatively associated with log of MBLL (Table-4). In multivariate analysis, *chapati* and plain biscuits were negatively associated with log of MBLL while *paratha* (wheat flat bread made with added fat) and vegetables cooked without potato were positively associated with log of MBLL (Table-4).

Table-1: Socio-demographic and exposure characteristics of pregnant women in urban and rural areas in Pakistan. (n=103)

Socio-demographic Variable	Frequency (%)
Women's age	
Younger (up till 29 years)	66 (64.1)
Older (≥30 years)	37 (35.9)
Husband's age	
Younger (up till 29 years)	26 (25.2)
Older (≥30 years)	77 (74.8)
Women's education	
Educated (secondary and above)	34 (33.0)
Un educated (no education, madrasa, primary school)	69 (67.0)
Socioeconomic status	
High SES	30 (29.1)
Middle SES	41 (39.8)
Lower SES	32 (31.1)
Exposure related variables	**Frequency (%)**
Source of water	
Non-piped water source	43 (41.7)
Piped water source	60 (58.3)
Cooking fuel	
Natural gas	64 (62.1)
Biomass	39 (37.9)
Residence	
Urban	63 (61.2)
Rural	40 (38.8)
Husband's occupation	
Jobless	26 (25.2)
Manual labour	55 (53.4)
Lead related work	22 (21.4)
Ethnicity	
Urdu speaking	51 (49.5)
Sindhi	40 (38.8)
Others	12 (11.7)
***Surma* use**	
Never	42 (40.8)
Ever	61 (59.2)
***Kajal* use**	
Never	56 (54.4)
Ever	47 (45.6)
Intake of non-food item during pregnancy	
No	65 (63.1)
Yes	38 (36.9)

Table-2: Association of food items intake (food frequency questionnaire) with women's blood lead levels (n=103)

Univariate analysis			**Multivariate analysis**			
	Mother blood lead levels		***Model I (adR^2= 0.616)**		****Model II (adR^2 =0.689)**	
Food items	**β (90%CI)**	***p*-value**	**β (90%CI)**	***p*-value**	**β (90%CI)**	***p*-value**
Chapati	-0.20 (-0.09, -0.01)	0.04	0.20 (0.02,0.06)	<0.001	-	-
Paratha	0.06 (-0.02, 0.03)	0.55	-	-	-	-
Puri/ kachori	0.27 (0.04,0.17)	0.01	-	-	-	-
Naan	0.09 (-0.04,0.12)	0.37	-	-	-	-
Bread slice	0.33 (0.04,0.09)	<0.001	-	-	-	-
Bun	0.22 (0.02,0.12)	0.03	-	-	-	-
Rusk	-0.32 (-0.08, -0.03)	<0.001	-	-	-	-
Plain cake	-0.21 (-0.09, -0.01)	0.03	-	-	-	-
Plain biscuits	0.11 (-0.01,0.05)	0.26	-	-	-	-
Cream biscuits	-0.17 (-0.08, -0.00)	0.08	-0.14 (-0.06, -0.01)	0.04		
Boiled rice	0.53 (0.07,0.13)	0.00	0.35 (0.05,0.09)	<0.001	0.18 (0.01,0.06)	0.01
Khitchri	0.15 (-0.008,0.130)	0.15	-	-	-	-
Fried rice	-0.13 (-0.09,0.01)	0.19	-	-	-	-
Biryani/ pulao	0.02 (-0.05,0.07)	0.81	-	-	-	-
Cooked cereals	0.23 (0.03,0.18)	0.02	-	-	-	-
Whole milk	0.24 (0.01,0.07)	0.02	-	-	-	-
Low fat milk	-0.42 (-0.09, -0.04)	<0.001	-	-	-	-
Yogurt/ raita	-0.17 (-0.09, -0.00)	0.09	-	-	-	-
Lassi	-0.60 (-0.12, -0.08)	<0.001	-0.31 (-0.08, -0.03)	<0.001		
Homemade mil desserts	0.33 (0.13,0.37)	<0.001	-	-	-	-
Market milk desserts	-0.15 (-0.15,0.01)	0.14	-0.22 (-0.17, -0.05)	<0.001	-0.11 (-0.10, -0.00)	0.08
Fresh fruits	0.20 (0.01,0.07)	0.04	-	-	-	-
Tetra pak market juice	0.08 (-0.03,0.07)	0.44				
Raw vegetables	0.39 (0.07,.16)	.00	-	-	-	-
Green leafy vegetables	0.17 (0.00,0.13)	0.08	-	-	-	-
Cooked root vegetables)	0.12 (-0.02,0.12)	0.21	0.16 (0.02,0.11)	0.03	-0.20 (-0.12, -0.01)	0.04

Univariate analysis			Multivariate analysis			
	Mother blood lead levels		*Model I (adR^2= 0.616)		**Model II (adR^2=0.689)	
Food items	β (90%CI)	*p*-value	β (90%CI)	*p*-value	β (90%CI)	*p*-value
Other vegetables cooked without potato	-0.19 (-0.11, -0.01)	0.05	-	-	-	-
Other vegetables cooked with potato	-0.21 (-0.10, -0.01)	0.03	-	-	-	-
Potatoes	0.36 (0.04,0.09)	<0.001	-	-	-	-
Beef	0.12 (-0.01,0.08)	0.22	-	-	-	-
Mutton	-0.09 (-0.08,0.02)	0.36	-	-	-	-
Chicken	0.11 (-0.02,0.07)	0.29	-	-	-	-
Fish	-0.30 (-0.18, -0.06)	<0.001	-0.16 (-0.11, -0.02)	0.02	-0.11 (-0.08, -0.01)	0.07
Egg fried	0.09 (-0.01,0.05)	0.32	-	-	-	-
Egg boiled	0.41 (0.05,0.11)	<0.001	-	-	-	-
Organ meat	0.29 (0.07,0.25)	<0.001	-	-	-	-
Daal	0.03 (-0.04,0.05)	0.76	-	-	-	-
Chole/ lobia	0.18 (0.01, 0.13)	0.07	-	-	-	-
Butter/ cream	0.13 (-0.01,0.07)	0.21	-	-	-	-
Fried savoury items	0.18 (0.00,0.11)	0.08	0.15 (0.01,0.08)	0.03		
Saltish savoury snacks	0.00 (-0.04,0.04)	0.10	-	-	-	-
Sweet snacks	0.31 (0.03,0.09)	<0.001	0.13 (0.00,0.05)	0.08	-	-
Supari/ gutka	0.15 (-0.00,0.06)	0.14	-0.13 (-0.04, -0.00)	0.06	-	-
Soft drinks	-0.25 (-0.11, -0.02)	0.01	-0.19 (-0.08, -0.02)	0.01	-0.14 (-0.06, -0.01)	0.02
Tea	0.21 (0.01,0.07)	0.04	-	-	-	-
Achar	0.06 (-0.04,0.09)	0.52	-	-	-	-

*Mutually adjusted; ** mutually adjusted and also with place of residence

Table-3: Association of intake of food items (food frequency questionnaire) with women's food lead levels (n=95)

Univariate analysis			Multivariate analysis			
			*Model I (adR2= 0.202)		**Model II (adR2=0.186)	
Food items	β (90%CI)	*p*-value	β (90%CI)	*p*-value	β (90%CI)	*p*-value
Chapati	0.07 (-0.02,0.05)	0.53	-	-	-	-
Paratha	0.03 (-0.02, 0.02)	0.77	-	-	-	-
Puri/ kachori	0.05 (-0.03,0.07)	0.61	-	-	-	-
Naan	-0.03 (-0.07, 0.05)	0.75	-	-	-	-
Bread slice	-0.01 (-0.03, 0.03)	0.93	-	-	-	-
Bun	0.02 (-0.03, 0.04)	0.87	-	-	-	-
Rusk	-0.04 (-0.03, 0.02)	0.69	-	-	-	-
Plain cake	-0.09 (-0.05, 0.01)	0.35	-	-	-	-
Plain biscuits	-0.15 (-0.05, 0.00)	0.16	-	-	-	-
Cream biscuits	0.01 (-0.03, 0.03)	0.94	-	-	-	-
Boiled rice	0.04 (-0.02, 0.03)	0.67	-	-	-	-
Khitchri	-0.07 (-0.07, 0.03)	0.51	-	-	-	-
Fried rice	-0.09 (-0.06,0.02)	0.37	-	-	-	-
Biryani/ pulao	-0.06 (-0.06,0.03)	0.57	-	-	-	-
Cooked cereals	0.05 (-0.04,0.07)	0.65	-	-	-	-
Whole milk	-0.02 (-0.03,0.02)	0.88	-	-	-	-
Low fat milk	-0.06 (-0.03,0.01)	0.57	-	-	-	-
Yogurt/ raita	-0.04 (-0.04,0.03)	0.69	-	-	-	-
Lassi	-0.16 (-0.04,0.00)	0.12	-0.21 (-0.05, -0.01)	0.04	-	-
Homemade mil desserts	0.00 (-0.09,0.09)	0.98	-	-	-	-
Market milk desserts	-0.13 (-0.11,0.01)	0.19	-0.20 (-0.14, -0.01)	0.05	-0.23 (-0.15, -0.02)	0.03
Fresh fruits	0.05 (-0.02,0.03)	0.61	-	-	-	-
Tetra pak market juice	0.21 (0.00,0.08)	0.04	0.30 (0.03,0.09)	<0.001	0.31 (0.03,0.10)	<0.001
Raw vegetables	0.03 (-0.030, 0.042)	0.79	-	-	-	-
Green leafy vegetables	0.09 (-0.02, 0.07)	0.38	-	-	-	-
Cooked root vegetables)	0.14 (-0.01, 0.09)	0.17	0.19 (0.01,0.10)	0.05	0.19 (0.01,0.10)	0.06
Other vegetables cooked without potato	0.07 (-0.02, 0.06)	0.48	-	-	-	-
Other vegetables cooked with potato	0.08 (-0.02, 0.05)	0.43	-	-	-	-
Potatoes	0.03 (-0.02, 0.03)	0.80				
Beef	-0.12 (-0.06, 0.01)	0.24	-0.16 (-0.06, -0.00)	0.09	-0.18 (-0.07, -0.00)	0.07
Mutton	-0.19 (-0.12, -0.01)	0.06	-0.21 (-0.12, -0.02)	0.03	-0.20 (-0.12, -0.01)	0.04
Chicken	-0.01 (-0.03,0.03)	0.96	-	-	-	-
Fish	-0.12 (-0.09, 0.02)	0.25	-	-	-	-
Egg fried	0.16 (-0.00, 0.05)	0.12	-	-	-	-
Egg boiled	0.05 (-0.02, 0.03)	0.65	-	-	-	-
Organ meat	-0.16 (-0.13, 0.00)	0.11	-0.30 (-0.18, -0.05)	<0.001	-0.31 (-0.19, -0.05)	0.01
Daal	0.01 (-0.03, 0.04)	0.96	-	-	-	-
Chole/ lobia	-0.11 (-0.07, 0.02)	0.29	-	-	-	-
Butter/ cream	0.02 (-0.02, 0.03)	0.82	-	-	-	-
Fried savoury items	-0.17 (-0.07, -0.00)	0.10	-	-	-	-
Saltish savoury snacks	-0.12 (-0.06, 0.01)	0.25	-	-	-	-
Sweet snacks	0.04 (-0.02, 0.03)	0.72	-	-	-	-
Supari/ gutka	-0.11 (-0.04, 0.01)	0.30	-	-	-	-
Soft drinks	-0.10 (-0.05, 0.01)	0.32	-	-	-	-
Tea	0.07 (-0.01, 0.03)	0.48	-	-	-	-
Achar	0.09 (-0.02, 0.08)	0.39	-	-	-	-

*Mutually adjusted; **mutually adjusted and also with place of residence

Table-4: Association of women food duplicate items with women's blood lead levels (standardized β-coefficient) (n=101)

Food items	β (90% CI)	*p*-value	β (90% CI)	*p*-value
Chapati	-0.45 (-0.09, -0.05)	<0.001	-0.32 (-0.07, -0.03)	<0.001
Paratha	0.51 (0.09,0.18)	<0.001	0.34 (0.05,0.13)	<0.001
Bread slice	0.05 (-0.07,0.12)	0.66	-	-
Rusk	-0.01 (-0.07,0.07)	0.95	-	-
Plain biscuits	-0.36 (-0.17, -0.07)	<0.001	-0.25 (-0.13, -0.04)	<0.001
Boiled rice	0.06 (-0.03,0.06)	0.57	-	-
Fried rice	-0.09 (-0.10,0.03)	0.34	-	-
Biryani/ pulao	0.27 (0.07,0.26)	0.01	-	-
Fresh fruits	-0.01 (-0.08,0.07)	0.94	-	-
Green leafy vegetables	-0.06 (-0.14,0.07)	0.54	-	-
Other vegetables cooked without potato	0.15 (-0.01,0.12)	0.14	0.13 (0.00,0.09)	0.09
Other vegetables cooked with potato	0.06 (-0.05,0.11)	0.56	-	-
Potatoes	-0.22 (-0.09, -0.01)	0.03	-	-
Beef	0.09 (-0.03,0.11)	0.34	-	-
Chicken	-0.00 (-0.09,0.08)	0.97	-	-
Fish	-0.11 (-0.28,0.06)	0.30	-	-
Fried egg	0.22 (0.03,0.22)	0.03	-	-
Boiled egg	-0.04 (-0.18,0.12)	0.71	-	-
Daal	0.25 (0.03,0.12)	0.01	-	-
Chole/lobia	0.03 (-0.11,0.14)	0.82	-	-
Saltish savory snacks	0.14 (-0.01,0.17)	0.16	-	-
Sweet snacks	0.00 (-0.08,0.08)	0.98	-	-
Tea	-0.08 (-0.08,0.03)	0.43	-	-

DISCUSSION

High blood Pb levels have been reported in several studies among vulnerable population in Pakistan.[16–18] Since, Pb has been controlled in petrol (the primary source), it was important to assess the contribution of secondary sources (particularly food) in elevating the blood Pb levels in relation to other pathways.[16] The current study reported the contribution of food in elevating the blood Pb levels in pregnant women. A recently linked case study from Karachi revealed that the body intake of lead (from soil, respirable dust and food) among pregnant women from different families ranged from 8.9–22.6 µg/kg body weight/week.[14] Among different exposures like food, air, dust, water and soil; food was the most important source of Pb intake among pregnant women. The contribution of Pb by food was higher for families with a higher exposure to Pb.[14]

We assessed the last one-month dietary intake of women through FFQ and their food duplicates were collected for three days. FFQ gave an insight into their usual dietary pattern and food duplicates gave the list of actual food items consumed by the study participants. It is the first study in Pakistan in which actual food duplicates were collected to determine the intake of Pb by mothers. Food duplicates is a very useful method to determine the actual food intake, different food items and their amount. There are studies in other parts of world in which food duplicates are done to determine the intake of nutrients and heavy metals by study participants.[18,19]

In this study, we found that the intake of animal products such as meat, milk and milk products were negatively associated with mother food and blood Pb levels. Calcium in milk has been reported to combat with Pb levels in bone, brain and nervous system. Calcium compete Pb for the same receptors in the body for absorption. It tends to occupy the calcium receptors in the body.[20]

From different food items, cooked root vegetables were found to increase the blood and food Pb levels among the study population. Wastewater irrigation of soil is an important factor in elevating heavy metals content in vegetables.[21] Irrigation of fields with wastewater in Pakistan is a cause of the heavy metal contamination of soil which is a serious concern to potential health impacts because of consuming contaminated products grown on these fields.[22,23] One study from Pakistan showed that the Pb contamination in soil and food crops irrigated with wastewater was more as compared to those irrigated with tube well water.[23] Soil samples were taken from different regions of a large city and then analysed for heavy metals content in them. It was found that the soil from agricultural, commercial and park areas had high concentrations of heavy metals including Pb.[24] Another important reason for positive association between Pb levels and cooked root vegetables could be the way of transport of these vegetables. These vegetables are transported without covering and are sold at road side with continuous exposure to the environmental pollutants.[25] Consumer attitude towards washing the vegetable before consumption may also be an important factor.[26]

Intake of boiled rice was positively associated with the MBLL both in urban and rural settings. Rice samples from Pakistan were reported to have high Pb content.[27] The Pb contamination of rice is reported from other studies conducted in South Asia.[28]

Tetra-pak market juices were positively associated with MFLL in both urban and rural settings. One study from Karachi collected different samples of packaged fruit juices and reported that they have heavy metals content in them including Pb.[29]

In our study, intake of fish was negatively associated with both MBLL and MFLL. However, one study from Karachi has reported that most of the fish types from Arabian Sea are contaminated with metals including Pb (30). The possible explanation could be that our study population was not taking the fish type which was reported to be contaminated with Pb. Eating *supari/gutka* and taking soft drinks are negatively associated with MBLL. This finding strength the evidence that the intake of such unhealthy food items decreases overall absorption of all nutrients by making a layer on intestinal wall.[31,32]

Strengths and Limitations: We comprehensively assessed the history of food intake and collected actual food duplicates for three days from households. We used a recall period of only last one month for FFQ so there should be good recall as generally a period of six months or one year is used by researchers.[33]

It was also noticed that some participants were not providing the exact food duplicates particularly avoiding the samples of expensive food items eaten by them which were mentioned in their food diary, which may have introduced some inaccuracies, though negligible. We assessed the BLL of study participants. Long-term exposure to Pb is best determined through measuring the bone Pb levels. But the method is expensive and inconvenient for the individual.

CONCLUSION

Dietary intake is an important contributor for high blood Pb levels. Commonly eaten food items identified in this study need to be separately analysed to measure their Pb content. Our results emphasize the need for involvement of food department, irrigation of food crops with treated water, transporting food with proper covering and educating consumers to wash food before eating. Overall, there is need for public awareness about the pathways and hazardous health effects of lead.

AUTHORS' CONTRIBUTION

SN acquired the data, analysed and interpreted it and drafted the manuscript. AZK gave input in FFQ, interpreted the results and critically reviewed the manuscript and finalized its version. AS and AG acquired and analysed the data. GR critically reviewed the manuscript and finalized its version. FK and ZF conceptualized and designed the study. ZF critically reviewed the manuscript draft and finalized its version.

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