

## Status of Toxic Metals in Biological Samples of Diabetic Mothers and Their Neonates

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**Abstract** The mechanism of transport of trace elements from the mother to the newborn is still not well known. The aim of present study was to compare the status of trace toxic elements, arsenic (As), cadmium (Cd), and lead (Pb) in biological samples (whole blood, urine and scalp hair) of insulin-dependent diabetic mothers (age ranged 30–40) and their newly born infants ( $n=76$ ). An age and socioeconomics matched 68 nondiabetic mothers and their infants, residing in the same locality, who were selected as referents. The elemental concentrations in all three biological samples were determined by an electrothermal atomic absorption spectrometer, prior to microwave-assisted acid digestion. The mean values of As, Cd, and Pb in all biological samples of diabetic mothers and their infants were significantly higher as compared to the referent mother–infant pair samples ( $p<0.01$ ). The high levels of As, Cd, and Pb in biological samples of diabetic women may play a role in the pathogenesis of diabetes mellitus and impacts on their neonates.

**Keywords** Diabetic women · Neonates · Heavy metals · Neonatal intensive care unit · Atomic absorption spectrophotometer

### Introduction

The deficiency of trace elements during intrauterine existence is closely related to mortality and morbidity in the newborns [1]. Since the transport of several essential elements across membranes is competitive, many divalent ions compete with each other, and positive or

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negative correlations between them may exist [2]. Toxic metal compounds are present in the human environment and have the potential for adverse health effects. Health risks are particularly associated with exposure in utero and the early years of life, since developmental and reproductive toxicity may occur even at low levels of exposure. Fetuses, newborns, and infants are developing organisms undergoing rapid changes in the structure and function of major organs and are therefore more vulnerable to the toxic effects of chemicals [3]. Risk assessment for the definition of acceptable or tolerable limits for chemicals in the environment must take into account the protection of the most vulnerable risk groups and consider the possibility of damage affecting the health of adults later in life and even future generations (e.g., damages to DNA or impairment of the reproductive function) [3]. Biomarkers are now recognized as essential tools for the evaluation of the extent and the effects of human exposure to chemicals [4]. Substantial differences exist between the physiology, metabolism, and lifestyle of young children compared to adults, which modify the patterns of exposure and limit the applicability of data obtained from the adult population [4]. Since prenatal life is the most sensitive human developmental stage, there is considerable interest in studying the toxic effects and deficiencies of trace elements in neonates. Measurement of trace element contents and their distribution in maternal and neonatal tissues may provide useful information on normal and abnormal levels during gestation and birth [3]. There is very little data on the ability of arsenic (As) to cross the placenta in man [2]. Arsenate has been shown to cross the placental barrier of the hamster, using both teratogenic doses and trace amounts [5].

Infants and pregnant women are more sensitive to toxic metals (TEs) [lead (Pb) and cadmium (Cd)] and hence more at risk than others. Negative effects on behavior and intelligence of children are found even at lower levels of TE exposure than those commonly associated with TE poisoning [6]. In general, TE accumulates in bones of the body and may be mobilized during a number of physiological and pathological conditions, e.g., age, osteoporosis, renal disease, pregnancy [6, 7]. Therefore, TEs stored in maternal bones can contribute to fetal Pb exposure [7]. There is no protective barrier to the transplacental transport of Pb and Cd during pregnancy; hence, fetuses are exposed to these substances [7]. Prenatal exposure to these metals, especially Pb, has been associated with adverse health effects [8].

In view of the above facts, it is important to determine the essential trace and toxic elements concentrations in biological samples of humans having physiological disorders such as psoriasis. Among various biopsy materials, serum, scalp hair, urine, and other body fluids may be used as bioindicators for these purposes [9–11]. Atomic absorption spectrometric methods are frequently used for the specific determination of very low elemental concentrations in biological samples [12–14]. At present, the mineralization methods that were frequently employed for the analysis of biological samples are wet digestion with concentrated acids using either convective systems or microwave ovens [15]. The main advantage of microwave-assisted samples pretreatment is its requirement of a small amount of mineral acids and a reduction in the production of nitrous vapors [15].

The aims of our study were to assess the status of trace toxic elements, As, Cd, and Pb, in the biological samples (scalp hair, blood, and urine) of diabetic mothers and their infants and compared the data with those obtained from referents (nondiabetic mothers and their infants) of matched age group. For comparative purposes, the biological samples of diabetic pregnant and nonpregnant, with referent healthy nonpregnant and pregnant of same age group, were also analyzed. The biological samples were prepared by microwave-assisted acid digestion method, and the validity of analytical procedure was checked by certified reference materials (CRMs) of all three biological samples as well as using conventional wet acid digestion on the same CRMs.

## Material and Methods

### Apparatus

The analysis of elements was carried out by means of a double-beam Perkin-Elmer atomic absorption spectrometer model 700 (Norwalk, CT, USA) equipped with a graphite furnace HGA-400, pyrocoated graphite tube with integrated platform, an autosampler AS-800, and deuterium lamp as background correction system and interfaced to a personal computer. The hollow cathode lamps of As, Pb, and Cd were run under the conditions suggested by the manufacturer. The experimental conditions for the determination of all elements were given in Table 1. Integrated absorbance signals computed by the AA spectrometer were employed throughout. A Pel (PMO23) domestic microwave oven (maximum heating power of 900 W) was used for digestion of the biological samples. Acid-washed polytetrafluoroethylene (PTFE) vessels and flasks were used for preparing and storing solutions.

### Reagents and Glasswares

Ultrapure water obtained from an ELGA LabWater (Bucks, UK) system was used throughout the work. All chemicals were of analytical reagent grade and were supplied by Merck (Darmstadt, Germany). Nitric acid  $\approx$  16 M and 30%  $H_2O_2$  were used for wet acid digestions. Standard solutions of As, Cd, and Pb were prepared by dilution of certified standard solutions of each (1,000 ppm) Fluka Kamica (Buchs, Switzerland). Dilute working standard solutions were prepared immediately prior to their use by stepwise dilution of the stock standard solution with 0.2 M  $HNO_3$ . Stock standard solution of Mg ( $NO_3$ )<sub>2</sub> (5.00 g/l), Pd (3.00 g/l), Pd 99.999% Sigma Aldrich, and ammonium phosphate ( $NH_4H_2PO_4$ ) Merck, used as a chemical modifiers, were prepared from Mg ( $NO_3$ )<sub>2</sub> Merck.

The mixture of magnesium nitrate and palladium were used for As, while Mg( $NO_3$ )<sub>2</sub> was used for Cd and Pb. All solutions were stored at 4°C until needed for analysis. Plasticware and glassware and the auto sampler cups were cleaned by soaking in 5 M

**Table 1** Measurement conditions for electrothermal atomization AAS 700

Parameters	As	Cd	Pb
Lamp Current (mA)	10.0	6.0	8.0
Wave length (nm)	193.7	228.8	283.3
Slit-width (nm)	0.7	0.7	0.7
Drying	140/15/15	140/15/5	140/15/5
Temp °C/ramp/hold(s)			
Ashing	1300/10/20	850/10/20	700/10/20
Temp°C /ramp/hold(s)			
Atomization	2300/0/5.0	1650/0/5.0	1800/0/5.0
Temp (°C)/ramp/hold (s)			
Cleaning	2600/1/3	2600/1/3	2600/1/3
Temp (°C)/ramp/hold (s)			
Chemical Modifier	Mg( $NO_3$ ) <sub>2</sub> +Pd( $NO_3$ ) <sub>2</sub>	Mg( $NO_3$ ) <sub>2</sub> +Pd( $NO_3$ ) <sub>2</sub>	Mg( $NO_3$ ) <sub>2</sub>

Sample volume (10  $\mu$ l), Cuvette = Cup, Carrier gas = (200 ml/min)

Background correction ( $D_2$  Lamp) used for all elements

HNO<sub>3</sub> for 24 h, rinsing five times with Milli-Q water, dried and stored in a class 100 laminar flow hood.

### Sample Collection and Pretreatment

The study protocol was approved by the Local Ethics Committee of Higher Education Commission of Pakistan. This was a hospital-based study, which was conducted among 68 healthy and 76 diabetic type 2 subjects with their infants, with age range 30–40 years. For comparison, we analyzed the biological samples of 93 referent nonpregnant women (RNPW), 89 diabetic nonpregnant women (DNPW), 115 referent pregnant women (RPW), and 75 diabetic pregnant women (DPW). All diabetic subjects used insulin therapy to maintain glucose metabolism. They were all urban residents of Hyderabad City, Pakistan.

The criteria for the diagnosis of diabetes mellitus was a positive glucose tolerance test, showing fasting blood glucose >140 mg/dl (>7.7 mmol/l) and postprandial blood glucose >200 mg/dl (>11.1 mmol/l), 2 h after 75 g of oral glucose. Only type-2 insulin-dependent diabetic patients were included in the study. The duration of diabetes among the patients was 10–15 years. A questionnaire was administered to all patients to collect details of their physical data, ethnic origin, duration of diabetes, insulin therapy, dietary habits, age, and consent. Physical examinations were performed at the Basic Health Unit (BHU) of Hyderabad City, Pakistan. At the start of the study, the participants' weight, height, blood pressure, and biochemical data were measured and recorded (Table 2). There were no statistically significant differences of height and weight between referents and diabetic patients. Blood samples were provided by the obstetric department of Liaquat Medical College Hospital in Hyderabad, Pakistan. Regarding delivery labors, in patients with normal blood glucose levels, 46 were normal, 12 were with forceps, and 10 were cesareans, while in diabetic patients, 38 were normal, 16 were with forceps, and 22 were cesareans. In referent group, 37 babies were male and 31 female and their weight ranged from 3.65 to 3.91 kg, while from the diabetic mothers, 39 were male and 37 were female and their weight ranged from 3.19 to 3.76 kg. All except two newborns from diabetic mothers were healthy.

Venous blood (3–5 ml) were sampled from understudy female subjects by using metal-free Safety Vacutainer blood-collecting tubes Becton Dickinson (Rutherford, NJ, USA) containing >1.5 mg K<sub>2</sub>EDTA. The samples were stored at –20°C until required for analysis. The blood samples were collected from the maternal vein and also from umbilical artery, and umbilical vein were collected after delivery, by clamping the umbilical cord.

In all understudy subjects, morning urine samples were collected in acid-washed, decontaminated 100 ml polyethylene tubes (Kartell, Milan, Italy), while samples of first urine of nascent baby were also collected. In-between sampling sessions, the container is wrapped in a clean polyethylene bag. Urine samples were acidified with ultrapure HNO<sub>3</sub> solution (1% v/v) and kept at –4°C. Prior to subsampling for analysis, the sample should be shaken vigorously for 1 min to ensure a homogeneous suspension.

The scalp hair samples (approximately 1.0 g each) were taken from five different parts of the scalp (frontal, cranial, occipital, right, and left lateral) of the newly born babies, while scalp hair samples of female subjects were collected from the nape of the neck. The scalp hair samples were washed and treated as reported in our previous study [16].

### Microwave-Assisted Acid Digestion

For digestion of biological samples, duplicate samples of each dried scalp hair samples (100 mg) were weighed directly into PTFE flasks (25 ml in capacity). For blood and urine,

**Table 2** Characteristics of Referent Nonpregnant Women (RNPW), Referent Mothers (RM), Diabetic Mothers (DM), Diabetic Nonpregnant Women (DNPW), Referent Pregnant Women (RPW), and Diabetic Pregnant Women (DPW)

Parameters	RNPW	RM	DM	DNPW	RPW	DPW
Height (cm)	156.2±2.1	149.2±1.3	155.6±0.7	155.5±1.2	159.5±2.3	156.6±1.5
BMI (kg/m <sup>2</sup> )	24.1±3.4	24.8±2.2 <sup>a</sup>	26.7±2.3 <sup>a</sup>	27.2±4.1	—	—
Weight (kg)	58.8±2.5	55.2±6.6	64.8±1.9	65.7±3.6	63.9±4.3	65.8±3.4
Pregnancy BMI (kg/m <sup>2</sup> )	—	25.3±1.1	27.9±0.9	—	23.4±2.2	29.6±3.2
Late pregnancy BMI (kg/m <sup>2</sup> )	—	30.2±2.2	27.9±0.9	—	29.1±2.5	35.1±2.6
Maternal net weight gain (kg)	—	7.6±4.1	8.6±3.2	—	6.5±1.2	7.4±2.5
Maternal insulin (μU/ml)	—	11.4±2.9 <sup>a</sup>	22.3±3.9 <sup>a</sup>	—	12.3±1.53	18.8±2.4
Maternal glucose (mg/dl)	—	85.6±5.9 <sup>a</sup>	122±12.4 <sup>a</sup>	—	92.8±2.7	139±8.87
Placenta weight (g)	—	459±42.5	635±34.8	—	—	—
Umbilical cord insulin (μU/ml)	—	11.6±4.9	12.8±4.2	—	—	—
Umbilical cord glucose (mg/dl)	—	61.5±9.42	72.7±10.3	—	—	—
Diabetes duration (year)	—	—	15	12	—	10
Waist circumference (cm)	63.5±1.1	64.4±5.3	76.6±5.2	68.2±2.5	65.3±2.3	72.3±3.5
Systolic blood pressure (mmHg)	112.3±4.9	118.2±5.3	129.8±6.6	121.8±3.2	114.5±5.1	127.6±5.6
Diastolic blood pressure (mmHg)	71.2±1.3	73.2±3.7	84.7±3.5	86.8±2.5	74.7±1.5	87.8±3.2
Glycohaemoglobin A1C (%)	4.97±0.68	5.36±0.55	8.11±0.22	8.42±0.36	5.89±0.72	9.26±0.47
Fasting plasma glucose (mmol/l)	(78, 86)	(82, 91)	(139, 185)	(132, 173)	(85, 93)	(147, 196)
Glycerol (μmol/l)	123±19.4	136±13.9	252±29.6	213±14.7	139±21	265±16.2
Glucose profiles at entry (mg/dl)	—	—	—	—	—	—
Mean fasting glucose	—	83.2±4.2 <sup>a</sup>	87.8±3.72 <sup>a</sup>	—	—	—
Mean postprandial glucose	—	115±11.6 <sup>a</sup>	121±9.73 <sup>a</sup>	—	—	—
Glucose profiles close to delivery (mg/dl)	—	—	—	—	—	—
Mean fasting glucose	—	81.4±6.97 <sup>a</sup>	83.5±7.49 <sup>a</sup>	—	—	—
Mean postprandial glucose	—	106±12.9 <sup>a</sup>	115±10.4 <sup>a</sup>	—	—	—
Fasting plasma insulin (mmol/l)	4.14	4.34	6.65	7.34	5.68	8.06

**Table 2** (continued)

Parameters	RNPW	RM	DM	DNPW	RNPW	DPW
Serum total cholesterol (mg/dl)	119±13	127±11	140±21	146±15	138±14	163±21
Serum HDL cholesterol (mg/dl)	42.3±3.6	45±4.1	27±7.3	34±1.2	43.6±5.6	29±2.8
Serum LDL cholesterol (mg/dl)	89±9.5	93±11	201±12	217±16	95.6±11.3	233±16.2
Free fatty acids (µmol/l)	126±22.5	138±19.4	252±35.8	237±18.6	128±12.7	286±23.7
Serum triglycerides (mg/dl)	135±15	144±39	259±32	211±25	158±19	274±22
Plasma albumin (g/dl)	3.8±0.7	3.6±0.8	3.1±0.6	3.2±0.4	3.3±0.5	3.2±0.3
Total plasma protein (g/dl)	6.9±0.7	6.7±0.6	6.2±0.5	6.1±0.4	6.5±0.5	5.6±0.6
Globulin (g/dl)	2.65±0.64	1.89±0.37	1.05±0.42	1.53±0.57	1.24±1.10	0.93±0.55
Immunoglobulin zst-units	245±45.6	213±36.4	95.6±29.3	148±39.5	186±68.3	87.2±25.3
Sodium (mmol/l)	144.5±1.3	138.5±3.2	134.4±1.5	135.4±1.2	142.3±2.5	121.5±1.1
Potassium (mmol/l)	5.3±0.8	4.6±0.8	3.7±0.6	3.5±0.3	4.8±0.5	3.1±0.5
Calcium (mmol/l)	2.1±0.2	2.3±0.3	2.2±0.2	2.3±0.3	2.0±0.4	1.9±0.3

<sup>a</sup> At the time of delivery

0.5 ml of each sample was directly taken into PTFE flasks. Added to each flasks was 2 ml of a freshly prepared mixture of concentrated  $\text{HNO}_3\text{-H}_2\text{O}_2$  (2:1, v/v) and kept for 10 min at room temperature, then placed the flasks in covered PTFE container. This was then heated following a one-stage digestion program at 80% of total power (900 W). Complete digestion of blood and urine required 2–4 min, while 5–8 min was necessary for scalp hair samples. After the required time intervals, the digestion flasks were cooled and the resulting solutions were evaporated to remove excess acid. Then 5 ml of 0.1 M nitric acid was added to the residue using a Whatman no. 42 filter paper and then diluted to 10.0 ml in volumetric flask. Blank extractions were carried through the complete procedure. Blanks and standard solutions were prepared in a similar acid matrix [17]. The validity and efficiency of the microwave-assisted digestion method was checked with certified samples of human hair, urine, and whole blood and also with those obtained from conventional wet acid digestion method [17]. The concentrations were obtained directly from calibration graph after correction of the signal obtained from blank. All experiments were conducted at room temperature (30–35°C) following well-established laboratory protocols.

### Statistical Evaluations

The statistical analyses were performed with the Excel X State computer program (Microsoft, Redmond, WA) and Minitab 13.2 (Minitab, State College, PA). The Student's *t* test was used to assess the significance of the differences in concentrations of elements among study subjects [18].

Calibration was performed with a series of As, Cd, and Pb standards. Sensitivity (*m*) was the slope value obtained by least-square regression analysis of calibration curves based on absorbance signals. The equation ( $n=5$ ) for the calibration curves was as follows:

$$\begin{aligned} Y &= (1.78 \times 10^{-1} \pm 9.9 \times 10^{-3})(\text{As}) + (2.5 \times 10^{-3} \pm 2.2 \times 10^{-3}), \\ Y &= (7.8 \times 10^{-3} \pm 3 \times 10^{-4})(\text{Cd}) + (9 \times 10^{-3} \pm 1.7 \times 10^{-2}), \\ Y &= (9 \times 10^{-4} \pm 2 \times 10^{-5})(\text{Pb}) + (7 \times 10^{-3} \pm 1.6 \times 10^{-3}), \end{aligned}$$

where *Y* is the integrated absorbance, and the concentration range of As, Cd, and Pb for calibration curve reached from the detection limits up to 100  $\mu\text{g/l}$ . The limits of detection (LOD) were 15.9, 1.62, and 24  $\mu\text{g/g}$  for As, Cd, and Pb, respectively, defined as 3 *s/m*. The quantification limits (LOQ) were defined as 10 *s/m*, calculated for As, Cd, and Pb as 56.8, 4.8, and 70.0  $\mu\text{g/g}$ , respectively.

## Results

### Biochemical Test

The biochemical results, as shown in Table 2, indicated that DNPW, DPW, and DM had higher prevalence of overweight and obesity ( $\text{BMI} > 25 \text{ kg/m}^2$ ), glycohemoglobin (%), fasting plasma glucose (mmol/l), fasting plasma insulin (mmol/l), serum total cholesterol, maternal insulin, maternal glucose, placenta weight, umbilical cord insulin, umbilical cord glucose, and hypertension (blood pressure  $> 140/90 \text{ mmHg}$ ) were higher as compared to RM, RPW, and RNPW, while plasma albumin and total plasma protein were lower ( $p < 0.05$ ). Immunoglobulins, globulin, and total plasma proteins decrease in women suffering from DM, DNPW, and DPW compared to RM, RNPW, and RPW ( $p < 0.05$ ). The DM also

had higher concentrations of plasma triglycerides and plasma LDL cholesterol, while lower concentrations of plasma HDL cholesterol was observed as compared to those in the RM ( $p < 0.001$ ; Table 2). The biochemical data of DM, DPW, and DW having type 2 diabetes show that the glucose level is not maintained properly.

Babies of DM mothers were heavier (3313.0 vs. 3420.0 g), but the difference was not significant ( $p < 0.05$ ), as compared to those born by RM. This difference did not remain significant when adjusted additionally for maternal BMI or height (Table 3). Our results are consistent with the literature in reported studies [19, 20]. Babies of DM mothers DM had higher body fat, fat mass, glucose, insulin, triglycerides, cholesterol, free fatty acids, and glycerol as compared to referent babies, but the difference was not significant ( $p < 0.05$ ; Table 3). Babies of DM mothers were longer, had larger mid arm, abdominal circumferences, subscapular and triceps skin fold thicknesses ( $p < 0.05$ ). There was no significant difference in the ponderal index and head and chest circumferences ( $p > 0.05$ ; Table 3). The DM group delivered 1–2 weeks earlier (36.2 vs. 38.3 weeks,  $p < 0.001$ ) than RM. There were no any apparent complications observed in neonatal of DM.

### Elemental Analysis of Biological Samples

The mean concentrations with standard deviations for each element in samples are shown in Table 4. The mean concentration of As in scalp hair samples of RM and their infants was significantly lower at 95% confidence interval [CI: 0.75, 0.83] and [CI: 0.45, 0.49]  $\mu\text{g/g}$  as

**Table 3** Anthropometric characteristics in babies of Referent Mothers (BRM) and Diabetic Mothers (BDM)

Parameters	BRM	BDM
Birth weight (g)	3313±337	3420±286
Birth weight, boys	3354±524	3554±435
Birth weight, girls (g)	3148±487	3373±367
Length (cm)	47.4±2.2	48.5±1.3
Ponderal index ( $\text{kg}/\text{m}^3$ )	3.11±0.5	2.99±0.4
Body fat (%)	11.9±1.35	13.5±2.65
fat mass (g)	359±136	432±136
Glucose (mg/dl)	69.2±23.2	77.9±19.8
Insulin ( $\mu\text{U}/\text{ml}$ )	11.3±4.33	12.6±3.64
Triglycerides (mg/dl)	42.3±19.4	45.4±15.1
Cholesterol (mg/dl)	62.6±16.2	65.4±17.9
Free fatty acids ( $\mu\text{mol}/\text{l}$ )	149.7±63.8	156.4±56.7
Glycerol ( $\mu\text{mol}/\text{l}$ )	86.4±32.8	91.6±49.2
Circumference		
Head (cm)	33.5±1.3	33.9±1.1
Chest (cm)	31.3±2.1	31.5±1.5
Abdomen (cm)	30.3±2.7	30.6±1.9
Midarm (cm)	10.5±1.0	10.9±0.7
Skinfolds		
Triceps (mm)	4.2±0.6	4.5±0.3
Subscapular (mm)	4.0±0.8	4.2±1.1



**Table 4** Trace element concentrations in biological samples (scalp hair, blood and urine samples) of Diabetic Mothers (DM), their infants, Referent Mother (RM) with their infants, Referent Pregnant (RPW) and Nonpregnant Women (RNPW) and Compared with Diabetic Pregnant (DPW) and Nonpregnant Women (DNPW)

	Arsenic			Cadmium			Lead		
	Scalp hair ( $\mu\text{g/g}$ )	Blood ( $\mu\text{g/l}$ )	Urine ( $\mu\text{g/l}$ )	Scalp hair ( $\mu\text{g/g}$ )	Blood ( $\mu\text{g/l}$ )	Urine ( $\mu\text{g/l}$ )	Scalp hair ( $\mu\text{g/g}$ )	Blood ( $\mu\text{g/l}$ )	Urine ( $\mu\text{g/l}$ )
	RM {68}	0.79±0.09	1.3±0.5	2.52±0.42	1.56±0.35	3.1±0.53	1.8±0.23	6.7±0.45	160±25.4
R <sup>I</sup> <sup>a</sup>	0.43±0.03	0.7±0.05	1.13±0.09	1.1±0.19	1.9±0.40	1.1±0.05	5.4±0.62	115±9.60	59.8±3.5
DM {76}	1.48±0.26	2.37±0.62	4.13±0.39	2.42±0.46	4.69±0.91	2.9±0.51	16.9±0.7	314±29.2	127±13.6
D <sup>I</sup> <sup>b</sup>	1.32±0.18	1.5±0.27	2.65±0.18	1.67±0.34	3.5±0.73	1.68±0.26	12.5±1.7	215±17.6	98.9±9.5
RPW {115}	0.92±0.12	1.25±0.4	2.63±0.33	1.42±0.26	4.2±0.64	1.96±0.21	7.2±0.8	196±12.3	79.6±6.3
DPW {75}	1.68±0.29	2.56±0.43	4.79±0.46	2.37±0.27	6.3±1.22	3.40±0.29	16.5±0.9	285±23.8	136.2±12.3
RNPW {93}	0.73±0.07	1.16±0.31	2.25±0.17	1.52±0.19	2.94±0.42	1.5±0.12	5.9±0.6	195±14.8	72.2±5.9
DNPW {89}	1.46±0.23	2.23±0.18	3.98±0.22	2.26±0.25	4.53±0.87	2.72±0.37	16.1±1.2	279±10.5	133.5±14.6

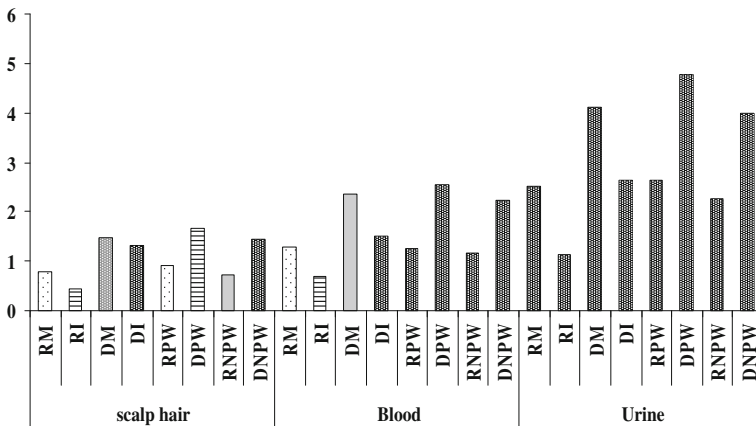
All results were calculated as mean with standard deviation ( $\bar{x} \pm \frac{st}{\sqrt{n}}$ )

R<sup>I</sup>/Referent infants, D<sup>I</sup>/diabetic infants

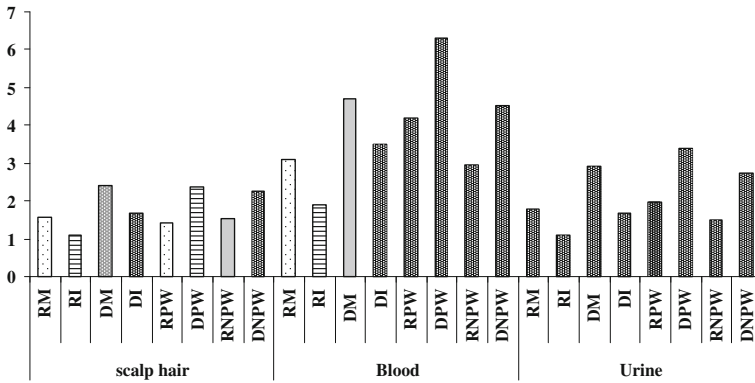
compared to DM and their new born baby [CI: 1.41, 1.56] and [CI: 1.25, 1.39]  $\mu\text{g/g}$ , respectively ( $p < 0.002-0.005$ ; Fig. 1). The level of As in blood of RM and their infants was found to be lower [CI: 0.93, 1.42] and [CI: 0.62, 0.72]  $\mu\text{g/l}$  than DM and their fetus [CI: 1.89, 2.63] and [CI: 1.36, 1.58]  $\mu\text{g/l}$ , respectively ( $p < 0.01$ ; Fig. 1). The excretion of As was higher in diabetic patients and their infants than that of the controls ( $p < 0.001$ ; Fig. 1). As regards neonatal and maternal blood and urine As, these were significantly lower to the figures of lactating women as reported by Concha et al. [21], which were 4.4–19 and 200–580  $\mu\text{g/l}$ , respectively. Our findings of As in blood of referents were consistent with the Sharma and Pervez’s [22] results of control mothers, who resides in the uncontaminated areas (0.35+0.1  $\mu\text{g/l}$ ).

Analysis of Cd in scalp hair revealed a significant difference between RM and DM ( $p < 0.01$ ). The mean concentration of Cd in the scalp hair samples of RM and their infants was found to be [CI: 1.25, 1.67] and [CI: 0.82, 1.33]  $\mu\text{g/g}$ , while the DM and their new born babies had [CI: 1.96, 2.72] and [CI: 1.38, 1.89]  $\mu\text{g/g}$ , respectively ( $p < 0.001-0.002$ ; Fig. 2). The scalp hair results of mothers and their infants are consistent with another study conducted in the contaminated areas, i.e., 1.45 and 1.27  $\mu\text{g/g}$ , respectively [23]. In blood samples, the Cd concentration levels in RM and their infants were found to be significantly lower [CI: 2.51, 3.34] and [CI: 1.34, 2.19]  $\mu\text{g/l}$  than the corresponding values of DM neonate pairs,  $p = 0.001-0.002$  and  $p < 0.001$ , respectively (Fig. 2). Elevated Cd was observed in urine samples of DM and their infants ( $p < 0.005$ ; Fig. 2). Our results of blood are consistent with other studies, whose subjects are residents in the uncontaminated areas (0.75+0.2  $\mu\text{g/l}$ ) [19] and (0.7  $\mu\text{g/l}$ ) [24].

Our research showed that the mean values of Pb in scalp hair and blood samples of RM and their neonates was significantly lower than DM infants ( $p = 0.001-0.004$ ; Fig. 3). The scalp hair results of mothers and their infants are consistent with other studies conducted in the uncontaminated (6.0 and 5.3)  $\mu\text{g/g}$  and contaminated areas (13.3 and 7.2)  $\mu\text{g/g}$ , respectively [23]. Our results of maternal and infants blood Pb are consistent with other studies, i.e., Tabaku et al. [25] (106, 89  $\mu\text{g/l}$ ) and Saxena et al. [26] (194, 170  $\mu\text{g/l}$ ), while higher as compared to Al-Saleh et al. [27] (55, 41  $\mu\text{g/l}$ ), Vasilios et al. [28] (37, 20  $\mu\text{g/l}$ ),



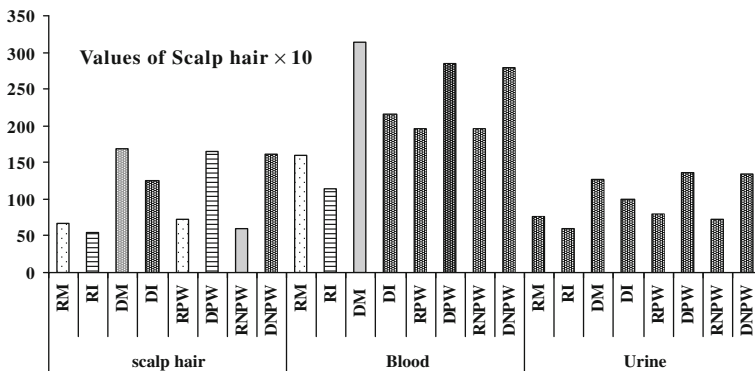
**Fig. 1** As concentrations in the scalp hair, blood, and urine samples of referent mother (RM) with their infants (RI), diabetic mothers (DM) with their infants (DI), referent pregnant (RP) and nonpregnant (RNPW) and compared with diabetic pregnant (DPW) and nonpregnant women (DNPW)



**Fig. 2** Cd concentrations in the scalp hair, blood, and urine samples of referent mother (RM) with their infants (RI), diabetic mothers (DM) with their infants (DI), referent pregnant (RP) and nonpregnant (RNPW) and compared with diabetic pregnant (DPW) and nonpregnant women (DNPW)

Lagerkvist et al. [29], (26, 22 µg/l), and Raghunathu et al. [24] (64, 51 µg/l). The levels of Pb were found to be higher in urine samples of DM and their infants than referents ( $p < 0.015-0.02$ ; Table 4; Fig. 3).

For comparison, we also analyzed the biological samples of healthy referents and diabetic pregnant and nonpregnant women. The level of As in the scalp hair samples of referent nonpregnant (RNPW) and pregnant women (RPW) was found to be lower at 95% confidence limit [CI: 0.65, 0.77] and [CI: 0.85, 0.99] µg/g, respectively, than diabetic nonpregnant women (DNPW) and diabetic pregnant women (DPW) [CI: 1.37, 1.53] and [CI: 1.62, 1.79] µg/g, respectively ( $p < 0.005$ ; Table 4; Fig. 1). The concentration of As in blood samples of DNPW and DPW was found to be lower [CI: 2.32, 2.69] and [CI: 2.03, 2.35] µg/l, as compared to RNPW and RPW [CI 0.96, 1.28] and [CI: 1.05, 1.39] µg/l, respectively ( $p < 0.01$ ; Fig. 1). The excretion of As was higher in diabetic patients than that of the controls ( $p < 0.011$ ; Table 4; Fig. 1). The concentration of Cd in scalp hair samples of



**Fig. 3** Pb concentrations in the scalp hair, blood, and urine samples of referent mother (RM) with their infants (RI), diabetic mothers (DM) with their infants (DI), referent pregnant (RP) and nonpregnant (RNPW) and compared with diabetic pregnant (DPW) and nonpregnant women (DNPW)

RNPW and RPW were found [CI: 1.32, 1.66] and [CI: 1.29, 1.57]  $\mu\text{g/g}$ ; however, DNPW and DPW had [CI: 2.05, 2.45] and [CI: 2.17, 2.63]  $\mu\text{g/g}$ , respectively ( $p < 0.007$ ; Table 4; Fig. 2). The concentration of Cd in blood of RNPW and RPW was found lower at [95% CI: 2.63, 3.18] and [CI: 3.56, 4.29]  $\mu\text{g/l}$  as compared to DNPW and DPW [CI: 4.26, 5.38] and [CI: 5.86, 6.58] with  $p < 0.002$  and  $0.001$  (Fig. 2). Higher Cd level was observed in urine samples of DNPW and DPW than RNPW and RPW ( $p < 0.004$ ; Table 4; Fig. 2). Analysis of scalp hair for Pb shows higher mean values in DNPW and DPW [CI: 15.3, 16.3] and [CI: 15.8, 16.9]  $\mu\text{g/g}$ , respectively, while that of RNPW and RPW had [CI: 5.36, 6.48] and [CI: 6.53, 7.34]  $\mu\text{g/g}$ , respectively ( $p < 0.002$ ; Fig. 3). In blood samples, the mean concentrations of Pb in RNPW and RPW subjects were found to be lower, [CI: 182, 206] and [CI: 185, 209]  $\mu\text{g/l}$ , than those obtained for Pb in blood samples of DNPW and DPW [CI: 268, 291] and [CI: 273, 298]  $\mu\text{g/l}$ , respectively ( $p < 0.034$ ; Table 4; Fig. 3). The levels of Pb were found to be higher in urine samples of DNPW and DPW than referents ( $p < 0.001$ – $0.002$ ; Table 4; Fig. 3).

The correlation of trace elements (As, Cd, and Pb) between RM and DM and also among RNPW and DNPW were statistically analyzed by multiple linear regression equation and Pearson correlation, as shown in Table 5.

The unpaired Student's  $t$  test at different degrees of freedom between all studied groups was calculated at different probabilities. Our calculated  $t$  value exceeds that of  $t$  critical value at 95% CIs, which indicated that the difference between the mean values of all three essential elements in normal and diabetic mothers showed significant differences ( $p < 0.001$ ).

## Discussion

The present study provides data on toxic element (As, Cd, and Pb) concentrations in scalp hair, blood, and urine obtained from the diabetic mothers and their infants and compared the data with those obtained from referents (nondiabetic mothers and their infants) of matched age group living in Hyderabad, Sindh, Pakistan. For comparative purposes, we analyzed the biological samples of referent and diabetic pregnant and nonpregnant women. It was observed that there is no significant difference in elemental concentration in the biological samples of infants of DM and RM; our results are consistent with another study which indicated that the levels of these elements in maternal blood are not useful indicators for the assessment of fetal growth [19, 30]. This factor in the infant is not only a major cause of stunting in childhood and adulthood but will also lead to increased risks for adult chronic diseases [31]. Some of these risks can either be enhanced or diminished in low birth weight (LBW) infants, depending on feeding patterns during the first year.

The importance of female nutrition precedes and extends beyond the reproductive years to optimize completion of adolescent growth and establish body nutrient reserves before pregnancy and to maintain adequate nutritional status, particularly skeletal health, through the postmenopausal years. Hence, the need for a constant, balanced, and adequate supply of all essential nutrients should be fulfilled lifelong to optimize both woman's and her offspring's health [1]. This is best achieved safely through a consistent balanced intake of nutrient-rich food including fortified foods, nutrient supplements whenever necessary, and regular exercise [32].

In the present study, we have shown that diabetic mothers and their newborns have higher levels of As, Cd, and Pb than normal mothers and their newborns. Nevertheless, the high levels of those toxicants found in the diabetic mothers and their newborns were not

**Table 5** Linear regression and pearson coefficient for As, Cd, and Pb concentrations in biological samples (scalp hair, blood, and urine samples) of Referent Nonpregnant Women (RNPW) vs. Diabetic Nonpregnant Women (DNPW) and Referent Mother (RM) vs. Diabetic Mother (DM)

	Arsenic			Cadmium			Lead		
	Scalp hair	Blood	Urine	Scalp hair	Blood	Urine	Scalp hair	Blood	Urine
	RNPW vs. DNPW	$0.47x+1.115$ $r=0.39$	$-0.21x+2.47$ $r=0.43$	$0.55x+2.75$ $r=0.46$	$0.49x+0.76$ $r=0.34$	$-1.33x+8.4$ $r=0.62$	$-1.4x+4.8$ $r=0.35$	$-1.05x+22.4$ $r=0.32$	$0.26x+230$ $r=0.41$
RM vs. DM	$-0.135x+2.11$ $r=0.45$	$-0.31x+11.2$ $r=0.54$	$-0.64x+2.21$ $r=0.56$	$-0.472x+2.9$ $r=0.41$	$-0.19x+72.8$ $r=0.57$	$-0.37x+21.3$ $r=0.58$	$-0.86x+9.8$ $r=0.49$	$-0.5x+63.9$ $r=0.42$	$-0.479x+2.46$ $r=0.45$

correlated to significant changes in oxidative stress parameters and/or antioxidant enzyme activities. Even though a clear source of contamination is unknown, diabetic mothers and pregnant women showing high levels of As, Cd, and Pb in their biological samples could have been exposed to major amounts of common environmental toxicants such as passive smoking and/or other combustion sources (cooking fires, industrial sources, air pollution, etc.). It has been documented that these sources may be a cause of exposure to carbon monoxide or particulates which themselves have been associated with fetal growth [33]. Fetal growth restriction could be related to impaired placental function (due to toxic metals), thus inhibiting appropriate transfer of essential nutrients to the fetuses, which are indispensable for life maintenance and normal development and growth [34].

There is rare information on Cd effects on insulin receptors and insulin action in adipose tissue [35]. In pancreatic islets of obese hyperglycemic mice, low Cd concentration evoked basal and glucose-stimulated insulin response [36]. In a previous report, we had shown that placental levels of Cd were inversely correlated to offspring birth weight [37]. In addition, high levels of Cd in placenta induced high levels of metallothionein (MT), a protein involved in the intracellular homeostasis of Cd and Zn [38]. Thus, additional MT not only retains Cd in the placenta to protect the fetus but may also affect fetus placental Zn dynamics, which, in turn, may lead to reduced Zn bioavailability to the fetus and thus contribute to weight impairment. Zinc is an essential micronutrient for fetal growth and for proper immune system function [39]. In the present study, Cd levels were clearly higher in diabetic mother's infants, and therefore the already described Cd/MT effect on Zn may be one of the factors contributing to LBW.

The results of this study support the association between a long-term As exposure and diabetes mellitus. The administration of As has been demonstrated to cause hyperglycemia in experimental animals and to affect the functions of insulin receptor and glucose transportation [40]. Arsenic has been found to cause mitochondrial damage, degeneration, and necrosis of  $\beta$  cells in the islets of mice after intraperitoneal injection of arsenite plus hydroxylamine, with a consequence of transient hyperglycemia [40]. Sulfhydryl groups play important structural and functional roles in both insulin receptors and glucose transporters [41]. Phenylarsine oxide, a trivalent arsenical, forms stable cyclic thioarsenite complexes with vicinal or paired thio groups of cellular proteins. This compound has been shown to inhibit glucose transport in adipocytes stimulated by the insulin mimickers vanadate and hydrogen peroxide [40]. Arsenic has been reported to interact with trace elements such as copper and zinc. Unfortunately, epidemiological data provides little or no insight as to how As exposure could adversely affect human pregnancies. These studies have shown that As exposure is linked to developmental defects and miscarriage [42]. Since placenta is a highly vascularized tissue, it has been hypothesized that As exposure causes placental dysmorphogenesis and defective placental vasculogenesis resulting in placental insufficiency and subsequent spontaneous abortion [43]. Hood [44] described the passage of inorganic As across the human placenta, in a mother ingesting a large quantity of As. This resulted in the death of the 30-week-old fetus. Accumulation of toxic metals does occur, although the precise mechanism is not clear [45].

Lead in blood is present almost entirely in the cells [46]. Infants are particularly sensitive to the toxic effects of Pb. A high percentage of the surviving children of exposed mothers suffer seizures and show signs of mental retardation [47].

Essential metal homeostasis should be crucial for the development of the fetus–placental unit due to their pleiotropic key role as cofactors in several enzyme systems [48]. One of the main defense mechanisms against effects of ROS is constituted by Se and Cu/Zn-dependent enzymes, glutathione peroxidase (GPx), and superoxide dismutase (SOD). It has been shown

that these enzymes may be inhibited by several heavy metals [49]. Therefore, the presence of heavy metals in placentas may be detrimental for placental GPx and SOD activities, and as a result, the fetus is subjected to some degree of oxidative stress which may result in potential damage [50, 51]. Studies related to transport of metals through the placenta have been discordant. On one side, it has been demonstrated that exposure and accumulation of specific metal contaminants during pregnancy is associated with deleterious effects to the offspring since the placenta is not an effectively heavy metal barrier. However, recent experiments performed in our laboratory showed that prenatal exposure to toxic metals induced accumulation of toxic metals in placentas. In the present study, the high accumulation of toxic metals could be associated to placenta damage, which could alter its selective permeability and functionality to essential nutrients [40]. Other studies have found positive correlations between levels of metals in blood of mothers and that of newborns, indicating transfer of these metals to the fetus through the placenta [52].

## Conclusion

The levels of understudy trace toxic elements are altered in insulin-dependent DM, DPW, and DNPW. The adverse long-term outcomes in the offspring may be associated with toxicity of As, Cd, and Pb in pregnancy. It was also observed that urban Pakistani women suffer from diabetes mellitus at the age of early 40 s. It was necessary to screen the diabetic pregnant women for efficacy of trace and essential metals to improve the outcome, both for the neonates and their mothers. Future studies should investigate these patterns in relation to the clinical courses of individual pregnancies in diabetic women, taking into account dietary supplements with essential micronutrients and medical treatments received during the pregnancy. The greater attention needs to be given for adequate supply of essential trace elements in diet of DM and diabetic pregnant women toward the possible ill-effects for the newborns. More detailed studies, however, are required to identify the potential negative effects of deficiency of essential trace elements in the pregnant woman and in normal and disease states. Further studies in this direction are in progress.

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**Conflicts of Interest** None.

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