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Toxic metals status in human blood and breast milk samples in an integrated steel plant environment in Central India

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Abstract

Owing to its unique nutritional and immunological characteristics, human milk is the most important food source for infants. Breast milk can, however, also be a pathway of maternal excretion of toxic elements. Selected toxic elements (As, Pb, Mn,a Hg and Cd) were determined in human breast milk and blood samples obtained from 120 subjects related to an integrated steel plant environment located in central India. Samples of breast milk and blood from subjects living outside the steel plant environment were also analyzed for comparative study. Higher levels of these toxic elements were found in blood samples as compared to breast milk samples. Plant workers showed the higher presence of these metals in their breast milk and blood samples compared to the residents of the area and the subjects living outside the industrial environment, respectively. Mn, Pb and Hg have shown a higher tendency to associate with blood and breast milk than As and Cd. The order of occurrence of these metals in blood and milk samples thus found is Mn > Pb > Hg > As > Cd.

1. Introduction

1.1. *Breast milk – the best source of infant nutrition*

Human milk is the best source of nutrition for infants. Breast milk contains the optimal balance of fats, carbohydrates, and proteins for developing babies, and it provides a range of benefits for growth, immunity, and development. Unfortunately, breast milk is not pristine. Contamination of human milk is widespread and is the consequence of decades of inadequately controlled pollution of the environment by toxic chemicals. The finding of toxic chemicals in breast milk raises important issues for pediatric practice, for the practice of public health, and for the environmental health research community (Landrigan *et al.* 2002).

It also illuminates gaps in current knowledge including: (a) insufficient information on the nature and levels of contaminants in breast milk, (b) lack of consistent protocols for collecting and analyzing breast milk samples, (c) lack of toxicokinetic data, and (d) lack of data on health outcomes that may be produced in infants by exposure to chemicals in breast milk. These gaps in information impede risk assessment and make difficult the formulation of evidence-based health guidance. To address these issues, there is a need for a carefully planned and conducted national breast milk monitoring effort in the developing countries such as India. Additionally, to assess health outcomes of toxic exposures *via* breast milk, it will be necessary to examine children prospectively over many years in longitudinal epidemiologic studies that use standardized examination protocols that specifically assess breast milk exposures. Finally, current risk assessment methods need to be expanded to include consideration of the potential risks posed to infants and children by exposure to chemical residues in breast milk (Landrigan *et al.* 2002).

1.2. *Pathways of exposure*

Pollutants enter the body through respiratory system, which consist of nasal cavity and tracheas known as upper respiratory system and bronchial tubes and lungs, which constitute lower respiratory system. Lungs contain several hundred millions small sacs called, alveoli which provide a surface area equivalent to 50 m^2 for exchange of gases. An average adult breathes approximately 30 pounds of air per day. The suspended particulate of specific size, range, and different compositions enter the body with inhaled air through nasal passage. Our respiratory system has a sound and effective defense mechanism, which collects and entraps most of the particles, before they could reach the alveoli of the lungs. This is accomplished with the help of motion of cilia lining the internal wall of respiratory tract and inertial implication of large size particulate at sharp bends in bronchial tree. In spite of this protection mechanism particulate between 0.1 and 1 μm are able to penetrate deep into the lungs. These particles contain various toxic metals like Pb, Cd, As and Hg. These metals are absorbed and accumulated in different parts of the body, disturbing the normal physiological functions (Environment Protection Agency, EPA 2002). The most common effect associated with metal toxicity is anemia, liver and kidney damage and nervous and gastrointestinal disturbances. Polycyclic aromatic hydrocarbons (PAHs) are found to be carcinogenic (Roosli 2001). Comparative measurement of lung deposition of inhaled fine particulate in normal subjects and patients with obstructive air-way disease has been reported earlier (Kim & Kang 1997).

1.3. *The study area*

The area studied is Steel City – Bhilai; the largest steel producing unit of India. In this area, different researchers have conducted several studies and have reported the mild contamination of air, water and soil with the pollutants generated by different manufacturing processes of the industry. Some toxic metals like Cr, Cu, Ni, Cd, Mn, etc. have been found in the settleable and suspended dusts in the vicinities of the same steel plant (Quraishi 1997). The presence of some selected toxic elements in various environmental samples like particulate matter, water and soil have also been reported (Sharma 2002). Status of toxic metals like Zn, Cr, Co, Ni, Cd, Pb, Hg, Cu, and Mn, in kidneys and gallstone samples of workers of the same steel plant have been reported (Pervez and Pandey 1994). Depositions of these toxic elements in the workers and residents of the same steel plant, thermal power plants and cement plant environment have also been reported (Quraishi and Pandey 1995). Absorption of Pb and Cd in different human body systems of both adults and infants indirectly by measuring their levels in ambient air of critically polluted areas such as Durgapur, Dhanbad and Asansol have also been reported by Mukharjee *et al.* (1997). Retention times of some very toxic elements like Pb, Cd, Cu and Zn in children's blood have also been reported as 20.3, 9.1, 2.3 and 2.3 days, respectively by Raghunath *et al.* (1997).

A number of potentially toxic metals have been reported in breast milk, including Pb, Hg and Cd (Rabinowitz *et al.* 1985; Radisch 1987; Grandjean *et al.* 1994, 1995; Oskarsson *et al.* 1995; Concha *et al.* 1998). Unlike the persistent organic pollutants, metals do not bind to the fat and so do not usually accumulate to higher concentration in breast milk than in blood (Golding 1997). As a result, infants are likely to be exposed to higher levels before birth than during breast feeding. Nonetheless, concentration of metals in breast milk is important for two reasons: first, as a pathway of exposure, and second, as an indicator of likely prenatal exposures (Oskarsson *et al.* 1998).

The work reported here describes estimations of selected toxic metals such as As, Pb, Mn, Hg and Cd in breast milk and blood samples obtained from 120 subjects in an integrated steel plant environment in central India (Figure 1). The subjects from whom the breast milk and blood samples collected included workers and residents of steel plant and steel plant township area, respectively. Estimations of samples collected from subjects living in uncontaminated areas were also carried out.

Fig. 1. Location of study area in central India.

2. Materials and methods

2.1. *Subjects*

The samples of human blood and breast milk from 120 subjects were collected from the Health Center of the steel plant township area during January–December 2001. The samples were collected on the basis of recorded medical data of the subjects concerned. The subjects were divided into categories of workers of the steel plant, and non-workers but residents of the steel plant township area. Some more subjects were selected for the collection of breast milk and blood samples from uncontaminated areas situated more than 100 km away from any industrial unit. The subjects were further divided into five age groups, 20–25, 25–30, 30–35, 35–40 and 40–45 years. Totally 40 samples were collected from workers category, 45 samples from non-worker but township residents category and 35 samples from uncontaminated category.

2.2. *Breast milk samples*

120 adult females were monitored during lactation up to 1 week from delivery. The samples were collected using a breast pump and expressed directly into precleaned polyethylene bottles. We were concerned that these samples might have been contaminated, but breast pump collection is a routine method used by Swedish investigators, with no apparent contamination (Hallén *et al.* 1995). Details of the maternal sampling and results of their blood, urine, and dietary intake have been published in several papers (Gulson *et al.* 1995, 1997, 1998).

2.3. *Blood samples*

Simultaneously, venous blood samples (10 ml each) were also collected from the same 120 adult females using sterilized syringes (Dispovan No. 24) and transferred to precleaned polyethylene bottles.

2.4. *Sample preparation*

Collected blood and breast milk samples were digested in 500 ml Pyrex Kjeldhal flask. 5 ml concentrated H_2SO_4 acid was added to it and heated over a small flame until most of the water was distilled off and the fluid began to char and spatter slightly. It was cooled for 2 min and added 2 ml of concentrated HNO_3 and boiled until brown fumes cease to evolve. This treatment was continued until the fluid did not char on boiling after evaluation of brown fume ceased. The content were cooled and 1 ml of HNO_3 and 1 ml $HClO_4$ (Perchloric acid) were added and boiled until most of the $HClO_4$ were volatilized. The final fluid on cooling was water clear (Welcher 1963).

2.5. *Determination of toxic metals*

The toxic metals (Pb, Mn, Cd, As and Hg) were determined in the digested samples of breast milk and blood using an atomic absorption spectrophotometer (Varian Techtron, Model Spectra AA 20BQ) following the recommended conditions of operations (Varian Publication 1989). Standard solutions for calibration purpose were prepared using spectroscopic grade compounds of the respective metals (Van Loon 1980). The samples

Table 1. Analysis data[a] of breast milk and blood samples for selected toxic metals around a steel plant environment in central India.

Category	Age group (years)	N	Blood	Milk	*r*	B/M ratio
A – Magnese content						
I	20–25	5	13.4 ± 7.5	4.6 ± 2.2	0.801	2.9
	25–30	10	28 ± 12.2	7.6 ± 4.8	0.687	3.7
	30–35	9	42.3 ± 18.8	12.3 ± 7.7	0.743	3.4
	35–40	12	73.6 ± 48.8	21.4 ± 4.6	0.587	3.4
	40–45	4	87.5 ± 60.2	24.5 ± 19.2	0.86	3.6
II	20–25	8	2.2 ± 0.8	0.6 ± 0.2	0.994	3.9
	25–30	7	4.1 ± 2.6	1.5 ± 1.0	0.754	2.8
	30–35	11	15.7 ± 6.3	4.3 ± 2.1	0.625	3.6
	35–40	9	18.3 ± 10.2	4.5 ± 1.8	0.256	4.1
	40–45	10	22.1 ± 14.0	5.7 ± 2.6	0.847	3.9
III	20–25	5	BDL	BDL	–	–
	25–30	6	0.3 ± 0.1	BDL	–	–
	30–35	10	0.5 ± 0.2	BDL	–	–
	35–40	9	2 ± 1.2	0.8 ± 0.1	0.213	2.5
	40–45	5	2.5 ± 1.1	1.5 ± 0.8	0.154	1.7
B – Lead content						
I	20–25	5	13.2 ± 5.8	3.6 ± 2.0	0.814	3.7
	25–30	10	32.8 ± 18.2	8.4 ± 3.3	0.852	3.9
	30–35	9	33.2 ± 26.3	11.5 ± 4.4	0.251	2.9
	35–40	12	52.5 ± 29.9	17.7 ± 12.2	0.756	3
	40–45	4	85.3 ± 21.7	22.3 ± 18.5	0.746	3.8
II	20–25	8	2.1 ± 0.9	0.6 ± 0.2	0.971	3.4
	25–30	7	4.2 ± 2.7	1.3 ± 0.4	0.765	3.2
	30–35	11	8.8 ± 4.0	3.1 ± 1.5	0.854	2.8
	35–40	9	14.3 ± 12.2	3.7 ± 2.5	0.988	3.9
	40–45	10	17.3 ± 10.2	4.2 ± 3.6	0.701	4.1
III	20–25	5	BDL	BDL	–	–
	25–30	6	0.2 ± 0.1	0.1 ± 0.0	0.452	2
	30–35	10	BDL	BDL	–	–
	35–40	9	0.5 ± 0.2	0.5 ± 0.2	0.765	1
	40–45	5	0.5 ± 0.3	0.7 ± 0.3	0.565	0.7
C – Mercury content						
I	20–25	5	5.1 ± 2.2	2.5 ± 1.1	0.921	2
	25–30	10	15.2 ± 11.1	6.7 ± 3.2	0.746	2.3
	30–35	9	16.8 ± 10.1	7.8 ± 4.3	0.847	2.2
	35–40	12	21.1 ± 18.5	12.3 ± 8.7	0.943	1.7
	40–45	4	31.5 ± 20.2	16.7 ± 11.1	0.074	1.9
II	20–25	8	0.8 ± 0.1	0.4 ± 0.1	0.985	2.1
	25–30	7	1.6 ± 1.0	0.9 ± 0.2	0.758	1.9
	30–35	11	5.8 ± 3.2	2.4 ± 1.9	0.986	2.4
	35–40	9	6.3 ± 5.3	3.2 ± 2.0	0.755	2
	40–45	10	2.8 ± 1.9	5.7 ± 4.1	0.986	0.5

Table 1. Continued.

Category	Age group (Years)	N	Blood	Milk	*r*	B/M ratio
III	20–25	5	0.3 ± 0.1	BDL	–	–
	25–30	6	BDL	0.1 ± 0.0	–	–
	30–35	10	BDL	BDL	–	–
	35–40	9	0.8 ± 0.2	0.9 ± 0.3	0.08	0.9
	40–45	5	0.9 ± 0.1	0.5 ± 0.3	0.754	1.8
D – Arsenic content						
I	20–25	5	1.6 ± 0.8	0.9 ± 0.2	0.847	1.9
	25–30	10	3.4 ± 2.3	1.7 ± 1.2	0.741	2
	30–35	9	6.2 ± 3.1	3.4 ± 2.3	0.777	1.8
	35–40	12	9.2 ± 4.8	4.5 ± 3.2	0.601	2
	40–45	4	10.8 ± 3.9	5.2 ± 3.8	0.91	2.1
II	20–25	8	0.9 ± 0.3	0.4 ± 0.1	0.858	2
	25–30	7	2.4 ± 1.9	0.9 ± 0.7	0.658	2.8
	30–35	11	3.7 ± 3.0	1.8 ± 0.3	0.766	2.1
	35–40	9	4.5 ± 2.2	2.3 ± 1.0	0.858	1.9
	40–45	10	6.6 ± 1.6	3.2 ± 2.2	0.258	2.1
III	20–25	5	0.1 ± 0.0	BDL	–	–
	25–30	6	BDL	BDL	–	–
	30–35	10	BDL	BDL	–	–
	35–40	9	0.2 ± 0.1	0.6 ± 0.1	0.456	0.3
	40–45	5	0.3 ± 0.1	0.9 ± 0.2	0.544	0.3
E – Cadmium content						
I	20–25	5	1.2 ± 0.3	0.6 ± 0.3	0.984	2.2
	25–30	10	3.8 ± 2.9	1.3 ± 0.7	0.747	2.9
	30–35	9	4.2 ± 3.4	2.3 ± 1.8	0.658	1.8
	35–40	12	6.8 ± 2.5	2.8 ± 2.0	0.557	2.4
	40–45	4	8.9 ± 5.6	3.8 ± 2.9	0.474	2.3
II	20–25	8	0.5 ± 0.1	0.3 ± 0.1	0.585	2.1
	25–30	7	1.8 ± 1.0	0.9 ± 0.3	0.687	2.1
	30–35	11	4.3 ± 2.3	2.1 ± 1.8	0.781	2
	35–40	9	3.8 ± 2.1	1.7 ± 1.0	0.625	2.2
	40–45	10	5.5 ± 1.9	2.3 ± 1.6	0.986	2.4
III	20–25	5	BDL	BDL	–	–
	25–30	6	0.1 ± 0.0	BDL	–	–
	30–35	10	0.1 ± 0.1	BDL	–	–
	35–40	9	0.5 ± 0.2	0.3 ± 0.1	0.754	1.7
	40–45	5	0.7 ± 0.3	0.1 ± 0.1	0.658	7

[a]Average of *N* measurements ± standard deviation values are given.

were suitably diluted to match the sensitivities of the standard solutions. The results obtained for Mn, Pb, Hg, As and Cd element are shown in Table 1(A–E).

3. Results and discussions

The results provide discernible patterns of accumulation of selected toxic metals in human blood and breast milk, showing distinct impacts of the environment to which the subject belonged. The data also show a marked difference in the accumulation of the toxic metals between the plant worker and non-worker related to the steel plant environment. The data obtained have a wide range of values, for example, 0.3–87.6 μg/l in case of Mn and 0.1–8.9 μg/l in case of Cd in blood samples while 0.8–21.5 μg/l in case of Mn and 0.1–3.8 μg/l in case of Cd in breast milk samples. In several cases, the levels of the toxic metals were below detection limit and the values are shown as BDL in the Table 1(A–E).

The plant workers showed a higher presence of metals as compared to the non-workers including resident category and uncontaminated subjects. The subjects related to steel plant and steel plant township area indicated a higher presence of toxic metals compared to uncontaminated areas, suggesting that the stack-emitted particles were contributory and potential carriers of the toxic metals. The presence of these toxic metals in ambient particulate matter in the same township area has also been reported by Pandey *et al.* (1998).

The uncontaminated subjects showed very small contamination of these toxic metals suggesting that the mineral handled in industries make a decisive contribution to accumulation in human blood and breast milk. The accumulation of these toxic elements in human blood and breast milk samples is found in the order of Mn > Pb > Hg > As > Cd. In all cases Mn, Pb and Hg showed a higher tendency to accumulate in breast milk and blood as compared to As and Cd. The accumulation of metals in blood and milk, in general found to be higher in older age group.

A higher concentration of these toxic elements is found in blood samples than in breast milk samples of the same subjects because unlike the persistent organic pollutants, metals do not bind to the fat and so do not usually accumulate in higher concentration in breast milk than in blood (Golding 1997). Correlation coefficient values between the blood and milk metal concentrations were also calculated for each age group. Good positive correlation coefficient values were obtained for category I and II but the similar relation is not maintainable in category III.

The ratio between the blood metal accumulation and milk metal accumulation is found to be different for individual metals. The accumulation of Mn in blood samples is four times higher than accumulation in breast milk. This ratio is calculated for each metal and presented as B/M ratio in Table 1(A–E). A similar pattern is obtained for lead. For Hg and As this B/M ratio is found to be 2. No significant B/M ratio is found for uncontaminated category.

A good positive correlation between the age group of the subjects and accumulation of toxic metals are found. Thus, breast milk is not the primary pathway of exposure for infants, prenatal transplacental exposure is a much greater concern. The said, instances of exposure through breast milk do occur, and are often important for an infant's total exposure. The presence of these toxic metals in ambient air of selected steel plant has already been reported. It is reasonable to infer that the steel plant environment is a contributory factor for the accumulation of these toxic metals in breast milk and blood of the subjects concerned.

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