Exposure to heavy metals among women, neonates and young children in Japan and Pakistan: food duplicate risk assessment study

[Pakistan component]

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Objectives:

- *To determine the total exposure of arsenic and lead among children, newborn and pregnant women in urban and rural population of Pakistan.*
    - *Determine the source and proportion of exposure for lead from food and air (source apportionment).*
- *To validate the food frequency questionnaire with the food group eaten by urban and rural population.*
- *To determine the biomarker of effects (searching for new biomarkers of effects).*
    - *Epigenetic difference in cord bloods due to exposure to heavy metals.*

Comparison studies:

- *To compare heavy metal (arsenic and lead) exposure (total intake) among urban and rural population in Pakistan.*
- *To compare the biomarkers of effects (as above) among and between Pakistani and Japanese population with the differential exposure.*

Introduction:

Metals and elements in food are of interest because of their potentials on human health risk. Some are known to be harmful to health (1). Lead is a known neurotoxin, particularly for young children. Its exposure during pregnancy and early childhood is harmful for neuropsychological development of the children (2). Inorganic arsenic is human carcinogen. Several non-carcinogenic adverse health effects of arsenic have also been identified (3, 4). Arsenic is not mutagenic, however affect the genes through epigenetic mechanism. While some other elements, such as copper, chromium, selenium and zinc are essential to health but they may be toxic at high levels of exposure. Heavy metals (arsenic and lead) exposure in food and also through air pollution lead to long term health effects among those exposed to these in their childhood. Lead is still the leading heavy metal with the highest global burden, especially for developing countries like Pakistan (5, 6). The underground water in Pakistan is heavy contaminated with Arsenic. The same water is used for agriculture and lead to further exposure through vegetable and fruits (7).

The health risk of heavy metals can be assessed by comparing estimates of dietary exposures with the Provisional Tolerable Weekly Intakes (PTWIs) and Provisional Maximum Tolerable Daily Intakes (PMTDIs) recommended by the FAO/WHO Joint Expert Committee on Food Additives (JECFA) as a part of the United Nations.

Malnutrition including under and over nutrition is an enormous public health problem worldwide, particularly in developing countries. Malnourished pregnant women and young children have devastating health effects in the shape of immediate and future burden of diseases. Malnutrition has several level of determinants from food production and agriculture to availability, cost and access to food, and also most importantly the choice and preparation/cooking of food. All of the above lead differential distribution of calorie intake, macro and micronutrient availability in the food.

Therefore, it is imperative to know the calorie intake, macronutrients distribution and its proportion in the local cuisine. Food frequency questionnaires (FFQ) are often used to determine the calories, macro and micronutrients in the food, based on recall. However, FFQ is subjective in nature and has its limitation in nutritional assessment (8). Food basket surveys and market basket surveys has also been done (9, 10). Also, even if the food types, amount and frequency is reliably recalled, the processing (cooking) of food itself make changes which are beyond assessment by FFQ and food basket surveys.

Therefore, it is important to have an objective assessment of calorie intake, macro and micronutrient intake, especially for pregnant women and young children. The information will not only do the objective assessment of intake of calories, macro and micronutrients among

pregnant and breastfeeding women and children in Pakistan but provide intervention options for improving nutritional status for the most vulnerable and important population subgroup where the burden of malnutrition is the largest.

Lead levels in environment and exposure is steadily decreasing in Pakistan. However, it is still very high from the health standards (11, 12). Millions of people living along river Indus are affected by arsenic through drinking groundwater. Total intake assessment of heavy metals such as arsenic in rural population and lead in urban areas among children and breastfeeding mothers in Pakistan will determine the future burden of disease among the population. All of this information may lead to policy formulation regarding food and heavy metal monitoring in Pakistan.

Materials and Methods:

Study site: The study will be conducted at two sites in Pakistan (and urban location in Karachi and a rural Gambat taluka, Khairpur, province of Sindh, Pakistan).

Karachi is a megacity and the population is exposed to high levels of lead due to air and soil pollution. The population living along River Indus is exposed to high level of arsenic through underground drinking water.

Study population: Three individuals of the same family will be recruited, including:

- *Women*
- *Newborn (1-3 months old), and*
- *Young child (2-5 year old)*

Sample size: A total of 100 families, 50 each from urban and rural location will be recruited for sampling. A small sample of 25-30 individuals, are appropriate for objective exposure assessment studies. Our sample of 50 at each location will also allow any subgroup analysis of high and low exposed population.

[Note: A total of 100 (50 each in Northern Japan – under Asahikawa Medical University - and Central Japan – Jichi Medical University) families will be recruited for comparative studies].

Samples and Sampling Methods:

1. Biological samples:
    - Hair samples: A sample 60-100 hairs from different places from the scalp base will be collected from the women for determining arsenic and lead exposure.
      Collection method: Hair samples will be collected by using gloves and will be kept in arsenic-free polyethylene bags with zip-lock before analysis.

- Nail samples: All nails of both palms and soles. We will determine arsenic and lead levels for exposure assessment.
  Collection method: Nail samples will be collected using gloves and kept in arsenic-free polyethylene bags with zip-lock before analysis.
- Placenta: It will be collected at the time of delivery. A sample of placenta will be cut (measuring 1x1 in diameter) from the fetal side and will be kept in polythene arsenic-free bags with zip-lock.
- Blood of pregnant women: 5ml blood will be collected by venipuncture.
- Cord blood for newborn: 8 ml blood will be collected from the cord.
- Blood of young child (sibling) (2-5 years old): 5 ml blood will be collected by venipuncture.
- Urine of breastfeeding women: Morning void urine samples of the breastfeeding women (60 ml) will be collected.
- Breast milk as newborn feed: One time breast milk sample of 30-50 ml will be collected for measurement of persistent organic pollutants (POPs), lead and arsenic.

2. Food duplicate samples:

   Food duplicates for three-days for the young child (2-5 year old), breastfeeding women and one-time breast milk (as food duplicate for newborn) will be collected from the family. 'Food duplicate' means the same amount of duplicate food which is eaten by the individuals.

   Collection method: Solid food will be collected for breakfast, lunch, dinner and snack in steel (arsenic and lead-free) containers separately for mother and child. Liquid food and water (total drinking water) will also be separately collected in plastic bottles for both child and mother.

3. Air and dust samples: Dust and air samples will be collected for 24 hours from the living rooms of the child (where the child spends most of his time in the home).
   Collection method: Dust will be collected for one day using vacuum cleaners. Air samples will be collected using low-volume samplers on 47mm glass filters.

4. Water sample: A sample of first-run tap or well water will be collected from the drinking water source for the household.

5. Lead contents in household items: Lead levels in household utensils, paint, dust, toys and furniture will measured using Niton handheld XRF analyzer. Spot samples will be

collected. Correlation between lead levels in household items and food lead and blood levels will be done.

Sampling procedures in a household:

A total three days will be spent and sampling will be done in one household (Day 0 to Day 2). An additional day (Day 3) will be kept for correction for any error in sampling. Sampling will be done on weekdays for three consecutive days. Sampling will not be done on any holiday.

Day 0: The sampling will start at 10:00 am in the morning. The monitoring time would be the same for each household. One-hour variation in start time is acceptable for different households. A total of 72 hours of drinks and food would be collected for the study subjects starting 10:00 am.

During the first visit, consent will be taken from the mother and assent for the child. The study objectives and sampling details will be explained to the mother. Mothers will be advised about the food diary and keeping records of food and drinks taken by her and the child. Sampling utensils will be provided to the mother with ice-box for keeping the food and drinking samples. Urine sample container will also be provided to the mother to collect it the following morning. Incentives for cooperation and for the provision of food duplicate will be explained to the mother and provided during the last visit.

Tap or well water samples (500 ml) will be collected in a separate container. Air sampling will be explained and air sampler will be installed during the first visit in the house for 24 hours.

Dietary History Questionnaire (DHQ) and Food Frequency Questionnaire (FFQ) will be administered to the mother and young child during the first visit.

Day 1: All food samples for Day 0 will be collected in the morning during the second visit and utensils will be provided for second day. Morning void urine sample will also be collected. Air samples and equipment will be collected from household. Food diary will be checked and completed with the help of the mother.

Day 2*:* All food samples for Day 1 will be collected in the morning during the third visit to household and utensils for Day 2 will provide. Food diary will be checked and completed with the help of the mother.

Day 3: All food samples for Day 2 will be collected in the morning during the fourth visit to the household. Food diary will be checked and completed.

Any error in sampling will be corrected by doing an additional day of sampling.

Sample measurement and processing (field and laboratory):

Weighing and measurement of food items: Solid food items will be categorized into common food groups and weighed. Liquid food intake will measured in liters.

Food sample processing: All food samples will be brought to a hired kitchen in ice-box and will be processed daily. All solid food will be broken into small pieces with the help of food processor and will be grinded using a grinder. [Note: Only eatable parts of the solid food will be grinded and the rest will be discarded. For example bones, seeds of fruits etc. will be discarded]. All liquid food will also be mixed with the solid food and grinded together. The whole sample will be poured into big mixture tank for homogenization. A sample of 35-40 ml will be collected in duplicate in sterile arsenic-free bottles, for Japan and Pakistan, after stirring the paste to make it homogenous.

Sample for freezing and dispatch: All samples will be kept frozen at -20 °C in a refrigerator before dispatch.

Laboratory analysis:

Laboratory analysis for As and Pb levels for all the samples will be conducted at Jichi Medical University in Japan.

Exposure measurement for arsenic (As) and lead (Pb) will be conducted using atomic absorption spectrophotometry (AAS) Non-radioactive isotope profiles of Pb in house dust and food duplicates and the biological samples from some subjects will also be analyzed for Pb source apportionment.

To assess body burden of As and Pb will be conducted by measuring the metal concentrations in the biological samples such as hairs, nails, blood, breast milk and urine. Whole blood and placenta samples will be processed to extract DNA using Qiagen kits.

Urine samples will also be analyzed for 8-OHdG concentration and Delta aminolevurinic acid concentration.

Statistical analysis:

The analysis will be done based on the following objectives:

- To determine the health effects due to arsenic and lead exposure
- To determine correlation between surma use of mother and child and blood lead levels of mother and child.

- To determine correlation between utensils, household dust, paint, toys and blood lead levels of mother and child.
- To determine the effect of arsenic and lead on child development (follow-up of the cohort using Bayley's scale).
- To determine the total sodium intake of young child, newborn and pregnant women.

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