AVAILABILITY OF ARSENIC IN BREAST MILK, EFFECT OF CHRONIC ARSENIC EXPOSURE ON TYPE 2 DIABETES, HYPERTENSION IN ADULTS AND ON CHILDREN'S NUTRITIONAL STATUS IN BANGLADESH.

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MBBS (Bangladesh), MPH (Australia)

A thesis submitted for the degree of Doctor of Philosophy in ‘Community Medicine and Clinical Epidemiology’
Under
The Center for Clinical Epidemiology and Biostatistics (CCEB)
School of Medicine and Public Health (SMPH)
Faculty of Health
University of Newcastle, NSW 2308
Australia

MARCH 2013
DECLARATION

This thesis contains no material which has been accepted for the award of any degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to the final version of my thesis being made available worldwide when deposited in the University’s Digital Repository**, subject to the provisions of the Copyright Act 1968. (**Unless an Embargo has been approved for a determined period.)

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“Also, I hereby certify that this thesis is submitted in the form of a series of published papers of which I am a joint author (1st author). I have included as part of the thesis a written statement from each co-author; and endorsed by the Faculty Assistant Dean (Research Training), attesting to my contribution to the joint publications.”

MD. RAFIQUL ISLAM
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LIST OF PAPERS TO SUBMIT AND PUBLISHED PAPERS

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DEDICATED TO

This thesis is dedicated to my deceased parents late. Mrs. Sayeda Islam and late Md. Nurul Islam and to those who are working for arsenic mitigation as individual or organization in Bangladesh and elsewhere and who are suffering from any adverse health conditions related to arsenic intoxication via drinking water; I firmly believe that our collaborative efforts will bring an easy and affordable mitigation strategy in near future.
**LIST OF ADDITIONAL PUBLICATIONS**

**CONFERENCE PRESENTATION**


**Published Abstract in the conference:**

**Title:** Association between Hypertension and Chronic Arsenic Exposure in Bangladesh.

**Objective:** To determine the association between chronic arsenic exposure via drinking water and prevalence of hypertension among rural Bangladeshi adults.

**Design, Setting, Participants:** This is an analytical cross sectional study among 1004 participants from 1682 eligible men and women (participation rate 60%) aged >30 years living in Bangladesh who had continuously consumed arsenic-contaminated drinking water for at least 6 months.

**Main Outcome Measures:** Hypertension was defined as systolic blood pressure > 140 mm of Hg or a diastolic blood pressure > 90 mm of Hg or in combination of the both.

**Results:** A total of 1004 individuals participated in the study. The prevalence of hypertension was 6.6% (95% CI 5.1-8.3%). After adjustment for participant’s age, sex, education, religion, marital status, sign of arsenical skin lesions, monthly household income and BMI, no excess risk of hypertension was observed for drinking water arsenic exposure over 50μg/L. Also, there was no increased risk for higher concentration of arsenic in the drinking water. Arsenic concentration >50μg/L in drinking water showed no association with systolic and diastolic hypertension separately however, it shows a strong relationship with increased pulse pressure when pulse pressure categorized as <55 and ≥55 mmHg (Adjusted OR: 3.06, CI: 1.22-7.65). Also, duration of exposure to arsenic did not show any impact on an increased risk to
hypertension except pulse pressure when exposed to arsenic for $\geq$10 years (Adjusted OR: 4.74, CI: 1.25-17.88).

**Conclusion:** Unlike other studies conducted in developing countries that reported a significant association, this study suggests no association between higher drinking water arsenic concentration and hypertension except for pulse pressure.
THESIS STRUCTURE

The thesis is comprises of two separate study outcomes. From the study project 1, we developed two manuscripts that are yet to publish (chapter 3 and 4) while from the study project 2, we developed two manuscripts (chapter 5 and 6) that are published in peer reviewed journals.

**Study project 1:** The longitudinal study on availability of arsenic in human milk and association between arsenic exposure and nutritional status in children living in arsenic contaminated areas in Bangladesh.

**Study project 2:** Analysis of previously collected data on association between chronic arsenic exposure and type 2 diabetes and hypertension in adults in Bangladesh

**Summary:**

The foremost part (chapter 1) describes the introduction of the two studies including literature review and research questions. Chapters 2 describes the detailed methodology of the longitudinal study conducted in an arsenic contaminated upazilla (sub-district) in Bangladesh among a cohort of 120 mother-infant pairs to determine the availability of arsenic in breast milk at 1, 6 and 9 months of child’s age and assessment of nutritional status at 3, 6 and 9 months of age of children living in this area. Chapter 3 and 4 are the outcomes of this longitudinal study.

The second and last part of the thesis (chapters 5 and 6) is based on data analysis for which the data were collected previously in a cross-sectional study among Bangladeshi adults and subsequently publication of papers in scientific journals. The cross-sectional study data were collected between January and July 2009 in Bangladesh to determine the association between chronic arsenic exposure and type 2 diabetes and hypertension in adults.
ABSTRACT

The discovery of extensive arsenic contamination of groundwater remains a public health concern in Bangladesh. Previous studies demonstrated a positive association with arsenic and numerous other diseases and health conditions including type 2 diabetes and hypertension in adults. There is also a growing body of concern on arsenic excretion via breast milk and the health consequences in children.

In the longitudinal study, we observed a low arsenic concentration in breast milk and the concentration was non-normally distributed and the median arsenic concentration in breast milk at all three time points (1, 6 and 9 months of child’s age) were remaining same as 0.5 µg/L. Arsenic in breast milk was non-significantly reduced over time (chapter 3). Arsenite (AsIII), arsenate (AsV), monomethyl arsenic acid (MMA), dimethyl arsinic acid (DMA) and arsenobetaine (AsB) were the constituents of total urinary arsenic; DMA was the predominant arsenic metabolite (approximately 70%) in infant urine at 1 and 6 months.

On the other hand, household’s arsenic exposure did not influence malnutrition in infants in the longitudinal study and the results were presented in chapter 4. Ten-percent infants at 3 month and 44% at 6 and 9 month of age were stunted irrespective of their levels of arsenic exposure. Underweight and wasting were the highest at 3 month. Overall wasting (<-2SD) was 23.3% at 3 month and no children were wasted at 6 and 9 month. While overall underweight (<-2SD) was 25% and 10% at 3 and 6 month respectively regardless of the households arsenic exposure levels. We observed differences in stunting at 9 months of age by arsenic exposure levels which might be due to a statistical incongruity and failure to establish differences in terms of other nutritional indicators such as wasting and underweight at other time points of infant’s age could be the resultant of a smaller sample size.
While in the data analysis project, we demonstrated a significant association between chronic arsenic exposure and type 2 diabetes. For most levels of arsenic exposure, the risk estimates are higher with longer exposure after adjusting all possible confounders; a dose–response pattern was also observed (chapter 5).

On the other hand, we failed to link an association between higher drinking water arsenic or duration with hypertension in the data analysis project (chapter 6) but observed an association with pulse pressure. Arsenic concentration as quartiles and >50 μg/L did show a strong relationship with increased pulse pressure, as did arsenic exposure for ≥10 years. Arsenic as quartiles showed a dose response relationship with increased pulse pressure.
Glossary

DNA    Deoxy-ribo Nucleic Acid
RNA    Ribo Nucleic Acid
µg/L    Microgram per liter
WHO    World Health Organization
As    Arsenic
As(III)    Arsenite or Arsenic in trivalent form
As(V)    Arsenate or Arsenic in pentavalent form
AsB    Arsenobetaine
AsH₃    Arsine gas
H₂    Hydrogen
MMA    Monomethylarsonic Acid
DMA    Di-methylarsinic Acid
PML/RAR    A fusion protein (RAR-Retinoic Acid Receptor)
BAMWSP    Bangladesh Arsenic Mitigation and Water Supply Project
D₂O    Deuterium Oxide or Heavy Water
CCEB    Centre for Clinical Epidemiology and Biostatistics
CHAD    Centre for Health and Development
ICMH    Institute of Child and Mother Health
T2D    Type 2 diabetes
BMRC    Bangladesh Medical Research Council
USA    United States of America
HCl    Hydrochloric Acid
HNO₃    Nitric Acid
NaBH₄    Sodium boro-hydride
KI    Potassium Iodide
FBG    Fasting blood glucose
Kg/m²    Kilogram per square meter
HbA1C    Glycosylated hemoglobin A1C or a type of hemoglobin
HREC    Human Research Ethics Committee
HAZ    Height for age Z scores or stunting
WAZ    Weight for age Z scores or underweight
WHZ    Weight for height Z scores or wasting
Coef:    Coefficient
BDHS    Bangladesh Demographic and Health Survey
FWA    Family Welfare Assistant
FPI    Family Planning Inspector
AFPI    Assistant Family Planning Inspector
IAEA    International Atomic Energy Agency
FTIR    Fourier transform infrared spectroscopy
GFAAS    Graphite furnace atomic absorption spectroscopy
POC bag    Point of care bag
HGAFS    Hydride generation atomic fluorescence spectroscopy
Km    Kilometer
mg/dl    Milligram per deciliter
HbA1C    Glycosylated haemoglobin A1C
SBP    Systolic blood pressure
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</tr>
<tr>
<td>FBG</td>
<td>Fasting blood glucose</td>
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<tr>
<td>mmHg</td>
<td>Millimeter of mercury</td>
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<tr>
<td>FIHGAAS</td>
<td>Flow injection-hydride generation atomic absorption spectrometry</td>
</tr>
<tr>
<td>SD</td>
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</tr>
<tr>
<td>OR</td>
<td>Odds ratio</td>
</tr>
<tr>
<td>Adj. OR</td>
<td>Adjusted odds ratio</td>
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<tr>
<td>CI</td>
<td>Confidence interval</td>
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<tr>
<td>NADPH</td>
<td>Nicotinamide adenine dinucleotide phosphate</td>
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<td>MnSOD</td>
<td>Manganese superoxide dismutase</td>
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<tr>
<td>NCD &amp; OPHI</td>
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<td>BMI</td>
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