Effects of early life arsenic exposure on the respiratory system, blood pressure and renal function in children and adolescents in rural Bangladesh, Matlab

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Acronyms

WHO	World Health Organization	ROS	Reactive Oxygen Species
UNICEF	United Nations Children's Fund	LDL	Low Density Lipoprotein
FVC	Forced Vital Capacity	CRP	C-Reactive Protein
FEV1	Forced Expiratory Volume in first Second	MAPK	Mitogen-Activated Protein Kinase
IRB	Institutional Review Board	β2MG	β2-Microglobulin
ISAAC	International Study of Asthma and Allergies in Childhood	eGFR	Estimated Glomerular Filtration Rate
ARI	Acute Respiratory Infection	PORs	Prevalence Odds Ratios
SBP	Systolic Blood Pressure	SMR	Standardized Mortality Ratio
DBP	Diastolic Blood Pressure	BMI	Body Mass Index
HDSS	Health and Demographic Surveillance System	GIS	Geographic Information System
MMA	Monomethylarsonic Acid	MMP9	Metaloprotease 9
DMA	Dimethylarsinic Acid	As	Arsenic
OR	Odds Ratio	iAs	Inorganic Arsenic
uAs	Urinary Arsenic	HR	Hazard Ratio

Chapter 1

Introduction

Arsenic is a metalloid with properties intermediate between a metal and a non-metal, and it is ubiquitously present in nature. Drinking water contamination with arsenic usually happens as a result of naturally occurring arsenic leaching into groundwater. Arsenic in drinking water is primarily present in inorganic form in the state of pentavalent arsenate (AsV) and trivalent arsenite (AsIII) (1). Arsenic contamination in well-water was first well known in Taiwan in the early 1960s (2). Millions of people across the globe are chronically exposed to arsenic concentration above the World Health Organization (WHO) guideline standard of 10µg/L (3) and this elevated level of arsenic has been detected in groundwater in nearly 70 countries in different regions around the world (4). Substantial exposure to arsenic in drinking water has been reported in many countries, including Argentina (5), India (6), Bangladesh (7), Chile (8, 9), China (10, 11), Taiwan (12, 13), Mexico (14), Vietnam (15, 16), and the USA (17-19).

Arsenic contamination of drinking water in Bangladesh is the unfortunate result of a successful programme of providing safe water to combat water-borne diseases like cholera and typhoid. In the 1970s, the United Nations Children's Fund (UNICEF) working with the Department of Public Health Engineering in Bangladesh drilled thousands of shallow tube wells to supply safe drinking water. Water from these wells is free from bacterial pathogens and was considered safe until the discovery of elevated levels of arsenic in well water in 1993 (20). However, the magnitude of the arsenic contamination in Bangladesh was highlighted nationally and internationally after the international conference on arsenic held in February 1998 in Dhaka (21). The British Geological Survey found that 27% of tested tubewells were contaminated with arsenic concentration above the country's limit of 50µg/l and nearly half of the wells exceeded the World Health Organization (WHO) standard of 10 µg/L. An estimated 35 million and 57 million people were exposed to arsenic via drinking water above the concentrations of 50µg/L and 10µg/L, respectively (22). Arsenic contamination in ground water in Bangladesh has been reported as the largest poisoning of a population in history (23). In 2002-03, the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) conducted a population based survey on 166,934 residents aged 5 years and above in rural Bangladesh, Matlab and water

from all 13,286 functional tubewells in that area were tested for arsenic concentrations. Over 60% of tubewells were found to be contaminated above the country's drinking water standard of 50 μg/l (24).

Metabolism of Arsenic in drinking water

Arsenic metabolism occurs through a series of methylation and reduction reactions. Methylation is considered the primary metabolic pathway of inorganic arsenic (iAs) in humans (25, 26). Once ingested, pentavalent arsenate is reduced to trivalent arsenite. Arsenite then undergoes an oxidative methylation forming monomethylarsonic acid (MMAv), which is reduced to monomethylarsonous acid (MMAIII). MMAIII is then methylated to dimethylarsinic acid (DMAv), which is reduced to dimethylarsinous acid (DMAIII).

Methylation of inorganic arsenic was considered to be a detoxification process (27) but the process is not complete in human. About one fourth of iAs remains unmethylated regardless of the exposure level (28). Recent evidence suggests the presence of intermediate reduced forms of the methylated metabolites, MMAIII and DMAIII in human urine (29-31). These trivalent intermediates especially MMAIII is more reactive and more carcinogenic (32, 33). However, MMAIII is highly unstable and rapidly oxidized to MMAV in urine, and is therefore extremely difficult to measure in field studies (34).

Urine is the primary route for elimination of inorganic arsenicals in human and almost all ingested arsenic is excreted through the urine. That's why the relative distribution of arsenic metabolites in urine is commonly used as a biomarker of arsenic methylation capacity (35). Typically, ingested iAs is excreted as 10–20% iAs, 10–15% MMA, and 60–75% DMA (28).

Early life arsenic exposure and health effects

Studies have shown that both inorganic arsenic and its intermediate toxic methylated metabolites can readily pass through the placenta and pregnant women exposed to arsenic via drinking water showed similar concentrations in the fetus as in the mother (36, 37). A study in Bangladesh has shown strong relationships between maternal and cord blood total arsenic As ($r = 0.93$, $p < 0.0001$). Cord blood arsenic metabolites ($n = 30$) were also significantly correlated with mother

blood arsenic: in dimethylarsinate (DMA) ($r = 0.94$, $p < 0.0001$), monomethylarsonate ($r = 0.80$, $p < 0.0001$), arsenite ($r = 0.80$, $p < 0.0001$), and arsenate ($r = 0.89$, $p < 0.0001$) (38). A study in Argentina suggested that children are more sensitive to As-induced toxicity than adults, as the methylated metabolites bind less to tissue constituents than inorganic As (39). A study has reported changes in arsenic metabolism throughout the pregnancy, might have deleterious effects on the developing fetus (40).

Evidence suggests that arsenic excretes in breast milk insignificantly. A study in Bangladesh has found low arsenic concentration in breast-milk (median, 1µg/kg; range, 0.25-19 µg/kg), in mothers exposed to high arsenic exposures via drinking water (10-1,100 µg/L in urine) (41, 42).

Early life exposure to arsenic has been linked to several and diverse health effects like adverse reproductive and birth outcomes, increased morbidity and mortality in childhood and development of non communicable diseases in adults including respiratory, cardiovascular, metabolic and kidney diseases, and various cancers (43-45).

Pregnancy Outcomes, Birth Weight and Infant Mortality

Exposure to arsenic in the critical developmental period in utero and early life has been implicated with negative reproductive outcomes, increased risk of morbidity and mortality in the neonatal period and during infancy (46-48).

Another study in Bangladesh also reported increased risk for spontaneous abortion and stillbirth for the women chronically exposed to arsenic concentrations of greater than >50 µg/L . The odds ratios for spontaneous abortion and stillbirth were OR=2.5; 95% CI=1.5–4.3) and 2.5 (95% CI =1.3-4.9) respectively (49).

Exposure to high concentrations of arsenic during pregnancy has been associated with increased risk of stillbirths. After adjustment for potential confounders, women exposed to arsenic concentration ≥ 200 µg/L during pregnancy had a 6-fold increased risk of stillbirth (Odds Ratio (OR) = 6.07, 95% confidence interval (CI): 1.54, 24.0; $p = 0.01$) (50).

A number of studies have reported on birth weight in relation to arsenic in drinking water. An ecological study in Chile reported a decline in average birth weight of 57 gms with an exposure level of less than 50 μg/L) (51).

A recent population-based prospective study involving 1,578 mother–infant pairs in rural Bangladesh reported an inverse association between prenatal arsenic exposure measured in urine and birth weight. In the range of maternal urinary arsenic concentrations below100 μg/L; birth weight reduced 1.68 gm for each 1 μg/L increase of arsenic in urine (52).

Excess infant mortality has been linked to prenatal exposure to arsenic in drinking water (50, 52). A study in Bangladesh reported increased risk of mortality in infants exposed to arsenic in early life. Infant death elevated with increasing arsenic exposure: the hazard ratio was 5.0 (95% CI = 1.4-18) in the highest quintile of maternal urinary arsenic concentrations (268-2019 μg /L; median = 390 μg/L), compared with the lowest quintile (<38 μg /L) (46).

Exposure to arsenic in early life and respiratory effect in children and adults

Two studies in Bangladesh have shown that prenatal exposure to arsenic increases the risk of acute respiratory tract infections in infancy (53, 54).

Several studies in Chile have reported various health consequences of early life arsenic exposure. Cohorts exposed to a very high concentration of arsenic (>800 μg/L) during in utero or early childhood have shown increased risk of respiratory mortality (43, 55). A study in Antofagasta, Chile found an association between early life exposure and increased risks of death from bronchiectasis. Subjects born just before the high-exposure period (1958-1970) and thus exposed during early childhood had a 12-fold increase in mortality (standardized mortality ratio [SMR] = 12.4; 95% confidence interval (CI) = 3.3–31.7), and the mortality rate was 46 times higher among those born during the peak exposure period (1950-1957) and therefore exposed in utero and early childhood (SMR= 46.2; 95% CI =21.1–87.7) (45). Early life exposure to high concentrations of arsenic has also been found to be associated with increased risk of respiratory symptoms and impairment in lung function. Subjects exposed to more than 800 μg/L in utero and or early childhood have nearly six-fold increase in breathlessness and around 12% (P=0.04) decline in FEV1 and FVC compared to the lowest exposure group (<50 μg/L) (56).

Based on the findings of early life arsenic exposure in Antofagasta, Chile, we planned a study in 2007 to investigate the respiratory effects in children 7-17 years exposed to arsenic in prenatal and early life. Using the 2002-2003 survey databases, we selected a cohort of 300 exposed children for whose mothers had the highest likely arsenic exposure during pregnancy, and another cohort of 300 unexposed children whose mothers' drinking water contained less than 10µg/l in early life. These two cohorts were invited to participate in the study to assess the effect of early life arsenic exposure on respiratory health including lung function in children. I directed the field work for this study which included getting lifetime drinking water histories and arsenic concentrations in the wells they used, detailed respiratory questionnaires, medical examinations, and measuring lung function. We found increased risk of respiratory symptoms but no significant decline in lung function (57). The children and adolescents exposed to arsenic water concentrations of more than 500 µg/L in utero had increased risk of respiratory symptoms, especially wheezing when not having a cold (OR=8.41, 95% CI: 1.66-42.6), and shortness of breath when walking fast or climbing (OR=3.19, 95% CI: 1.22-8.32). The full paper is given in Appendix 7.

Exposure to arsenic in early life and cardiovascular and renal effect in children and adults

Several studies have suggested that prolonged exposure to arsenic in drinking water increases the risk of cardiovascular and renal morbidity and mortality in adults (58-63). A few studies have investigated the effects of early life As exposure on cardiovascular and renal risk longitudinally. Yuan et al. showed that exposure to arsenic in prenatal and early life was associated with increased risk of death from acute myocardial infarction among adult males aged 30–49 years (44).

Hawkesworth et al. reported that children exposed to arsenic in utero showed a mild increase in blood pressure at the age of 4.5 years. Modest reduction in glomerular filtration rate has been observed in children aged 4.5 years exposed to arsenic in early life (-33.4 ml/min/1.72 m(2); 95% CI: -70.2, 3.34; P: 0.08) (64).

A set of autopsy reports from Chile suggested a link between prenatal and early life As exposure and cardiovascular-related diseases in young children (65).

A study analyzing mortality data among arsenic exposed children in Matlab, Bangladesh aged 5–18 years reported an elevated risk of mortality risks of childhood death from cardiovascular events or cancer (n = 22, HR: 2.18, 95% CI: 1.15–4.16) (66).

Increased death from chronic kidney disease has been reported among adults in Antofagasta, Chile exposed to high arsenic concentration (>800 µg/L) in utero or early childhood. Mortality was increased 2-fold [SMR=2.0; 95% Confidence Interval (CI): 1.5 to 2.8] in young adults aged 30-59 born before or during the high-exposure period of 1958–1970 (43).

Another study in Chile has reported heightened risk of death from renal cancer [SMR=7.1; 95% Confidence Interval (CI): 3.1 to 14] among people aged 30-39 years exposed to high concentration of arsenic in utero and/or early childhood (67).

Rationale

Several studies in Bangladesh and abroad have documented the association between long term inorganic arsenic exposure and non-malignant illnesses such as chronic respiratory diseases including decrements in lung function, hypertension and chronic kidney diseases in adults. Some studies investigated the effects of early life arsenic exposure on respiratory symptoms including lung function, blood pressure and renal function in early childhood or adults. However, no studies had looked at the impact of early life exposure in older children and adolescents.

For my PhD thesis, I intended to investigate the effects of early life exposure to arsenic by following a sub-cohort of 200 children of which half is highly exposed to arsenic (<400 µg/L) in early childhood.

I selected a sub-cohort of 110 most highly exposed children with an arsenic exposure level greater than 400µg/L in drinking water used by their mother during pregnancy and their early childhood. For each exposed child, one sex and age-matched unexposed child was selected who was not exposed to an arsenic concentration higher than 10µg/L during prenatal period and early childhood. We selected the highest and lowest exposure group to increase the power of the study. The objective was to assess respiratory, cardiovascular and renal effects in childhood and adolescence.

I was actively involved in all phases of the study; generating ideas, developing the proposal, seeking funding, obtaining Institutional Review Board (IRB) approval, developing consent forms and data tools, hiring and training of the field staff, supervising data collection at field level, data analysis and writing the thesis. I conducted an extensive literature review in which I studied hundreds of papers before writing the protocol. The study design was developed in collaboration with the University of California, Berkeley.

Chapter 2 describes the methods and design of the study; chapter 3 highlights the effects of early life arsenic exposure on the respiratory system; chapter 4 focuses on the association between in early life arsenic exposure and blood pressure in children and adolescents and chapter 5 concerns the impact of early life arsenic exposure on kidney function. Chapter 6 presents a brief summary and conclusion of the overall findings and conclusion.

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Chapter 2

Methods and study design

Overview of Study design

We conducted a prospective study of two sub-cohorts, one with high arsenic exposure in early life (107 children exposed to >400 µg/L of arsenic in drinking water), and the other with low exposure in early life (93 children not known to have been exposed in early life to more than 10 µg /L of arsenic). These sub-cohorts of children were originally identified from a total population survey of 166,934 residents in Matlab which included measurement of arsenic in their tube wells used for drinking water.

Study Area

The study was conducted in rural Bangladesh in Matlab which is a sub-district about 57 km southeast of Dhaka, the capital city of Bangladesh. Since 1966, the International Centre for Diarrheal Disease Research, Bangladesh (icddr,b) has been maintaining an internationally recognized and unique Health and Demographic Surveillance System (HDSS) in 142 villages in Matlab, covering a population of over 220,000. Vital events like birth, death, marriage, and in and out migration, are recorded by household visits every two months. Each inhabitant in Matlab HDSS area is assigned a permanent registration number and another identification number to locate his/her current residential location. If someone moves from one place to another inside Matlab HDSS area, the current identification number is updated accordingly. In case of outmigration, detailed information including contact phone number is recorded so that he/she could be located for follow-up. A geographic information system (GIS) is an integrated part of HDSS, and includes spatial information on households, tube wells, health facilities, and landscape characteristics. icddr,b has a central health facility and hospital and four sub-centres to provide health services and to support clinical and public-health research in Matlab.

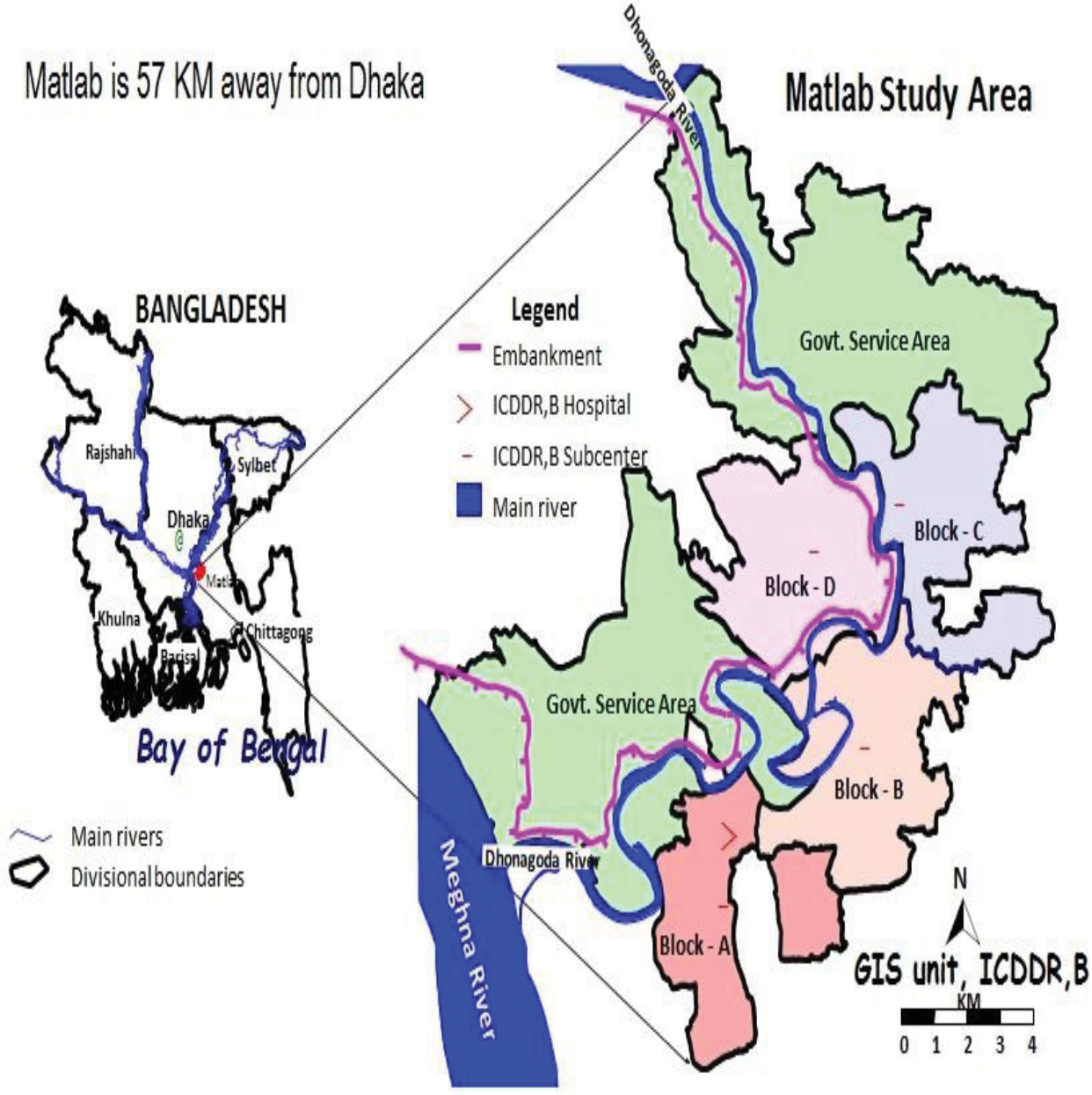

Fig 1: Map showing the study area in Matlab, Bangladesh

Selection of subjects

In 2002–03, icddr,b conducted a population-based survey of 166,934 subjects aged 5 years and above. Individual lifetime drinking water histories were obtained and all 13,286 functioning tube-wells, the primary sources of drinking water, were tested for water arsenic concentration. Tubewell arsenic concentrations ranged from <1 to 3644 µg/l. More than two-thirds of the tubewells exceeded the World Health Organization (WHO) recommended arsenic limit of 10µg/L, and more than 60% of tubewells contained arsenic above 50 µg/L, the Bangladesh standard (1).

A total of 52,797 children in Matlab were aged 7-17 years in the year 2008, and had arsenic measurements in their house drinking water source identified in the survey in 2002-2003. Names and addresses of children aged 7-17 years were abstracted from the Health and Demographic Surveillance System (HDSS) records and in prenatal exposure was assessed based on their mother's drinking water arsenic concentration during pregnancy. From this information, we selected a cohort of 300 exposed children for whose mothers had the highest likely arsenic exposure during pregnancy, and another cohort of 300 unexposed children whose mothers' drinking water contained less than 10µg/l in early life. These two cohorts were invited to participate in the original study to assess the effect of early life arsenic exposure on respiratory health in children (2). The investigation included detailed exposure assessment, identification of respiratory illnesses and measurement of lung function.

Flow chart of Study Participants Selection

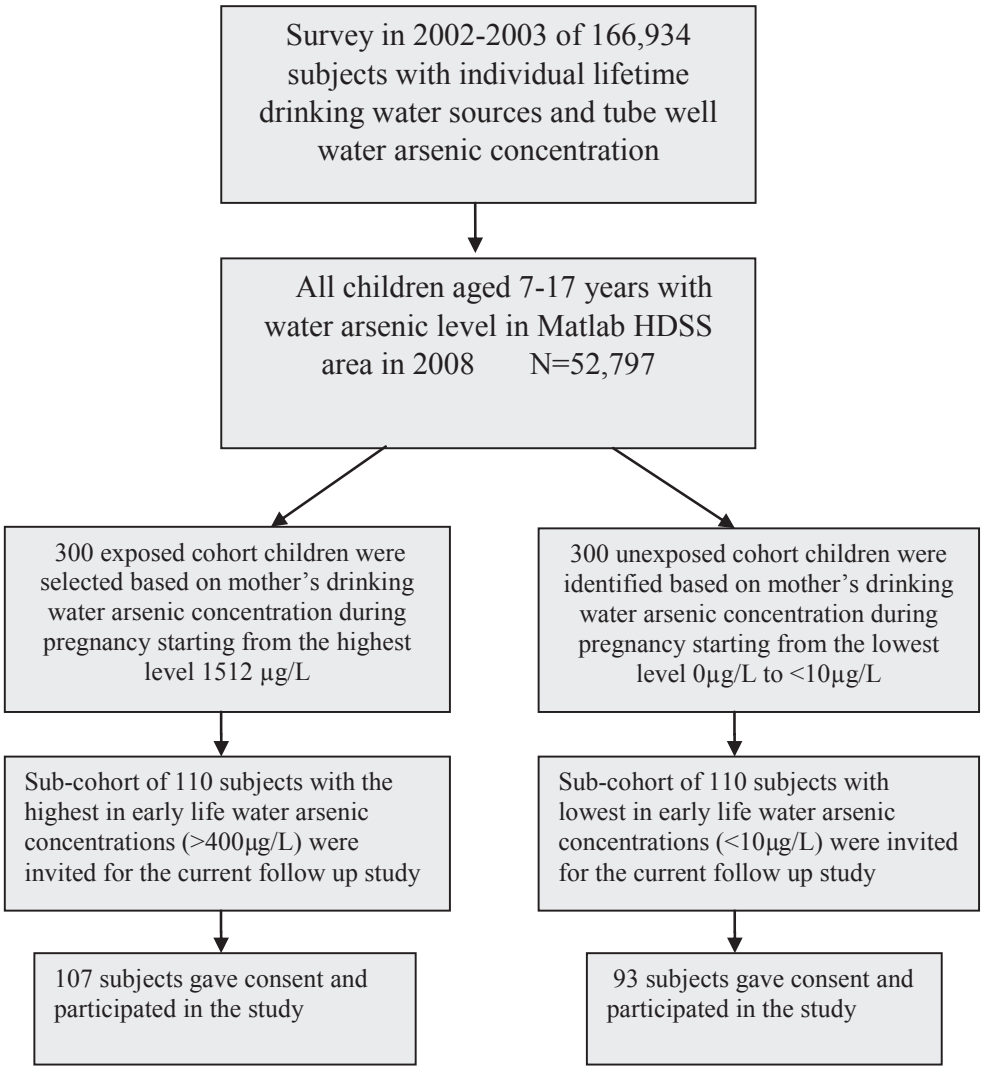

Fig 2: Flow chart showing selection process of study participants

Using the information of 600 children obtained in our previous study, we selected a sub-cohort of 110 most highly exposed children with in early life arsenic exposure greater than 400 μg/L. High exposure experience increases study power to detect new outcomes, and reduces the likelihood that effects could be due to confounding. For each exposed child, one sex and age-matched unexposed child was selected who was not exposed to an arsenic concentration higher than 10μg/L during prenatal period and early childhood. In total, 107 exposed and 93 unexposed children participated in the study.

In summary, my thesis consists of a prospective follow-up of a sub-cohort of 107 top most exposed adolescents and a sub-cohort of 93 adolescents with the lowest arsenic exposure in early life.

Arsenic Exposure Assessment

Children's lifetime drinking water histories and arsenic concentration measurements in all tube wells used since in utero were recorded. Details of early life exposure assessment were described in our earlier study (2) (Appendix-7). In brief, children and their mother were asked to provide the location of houses that they had lived in for at least six month, starting from nine months prior to the child's birth and ending with the house they had been residing in during our last visit during 2008-10. Then mothers were asked to identify their primary and secondary drinking water sources at home and the children were asked if they drank water at school. Water arsenic concentrations of all functioning tube wells used at home and school for at least six months were measured. In utero arsenic exposure was assessed based on arsenic concentration in the tube wells used by the mother throughout pregnancy. Postnatal exposure for the first five years was calculated by taking the average water arsenic concentration during the first five years of life. Well water arsenic concentrations were weighted by the proportions of water used by a child from each different water source. For tube wells from which we could not collect water samples, the arsenic concentrations measured in these wells during the 2002-2003 survey in Matlab were used. Some participants had occasionally used pond or river water for drinking. Since arsenic concentrations in pond/river water samples were very low or non-detectable, we used zero for pond/river water sources.

In our further follow-up of the two sub-cohorts of children, our interviewers visited the homes to update the drinking water history since our last visit. Firstly, mothers were asked about house changes and if they moved to new houses, they were asked to provide the location of houses that they had lived in at least six months. They also were requested to show their primary drinking and secondary water sources at new residences. If there was no residential change, any change in water sources was recorded. School water history was also augmented in a similar manner. Water samples from all the available new sources at home and at school were collected. Water specimens were taken in duplicate in 20 ml hydrochloric acid-washed vials following pumping the well for 30 strokes. Acid was added in the container to prevent precipitation of iron and co-precipitation of arsenic (1). They were kept at –20°C in Matlab and transported to the icddr,b Dhaka laboratory for analysis. Arsenic concentrations were measured by hydride generation atomic absorption spectrophotometry (HG-AAS, Shimadzu Model AA-6800 (3). The limit of detection (LOD) was 1 µg/l and non-detectable concentrations were assigned a value of 0.5 µg/l.

To obtain histories of respiratory and other medical illnesses, physical examination, blood pressure measurement, lung function tests and biological specimens, participants were invited to visit the Matlab hospital run by icddr,b. The mother or main care taker was requested to accompany the child to the hospital.

Household location of study participants in Matlab

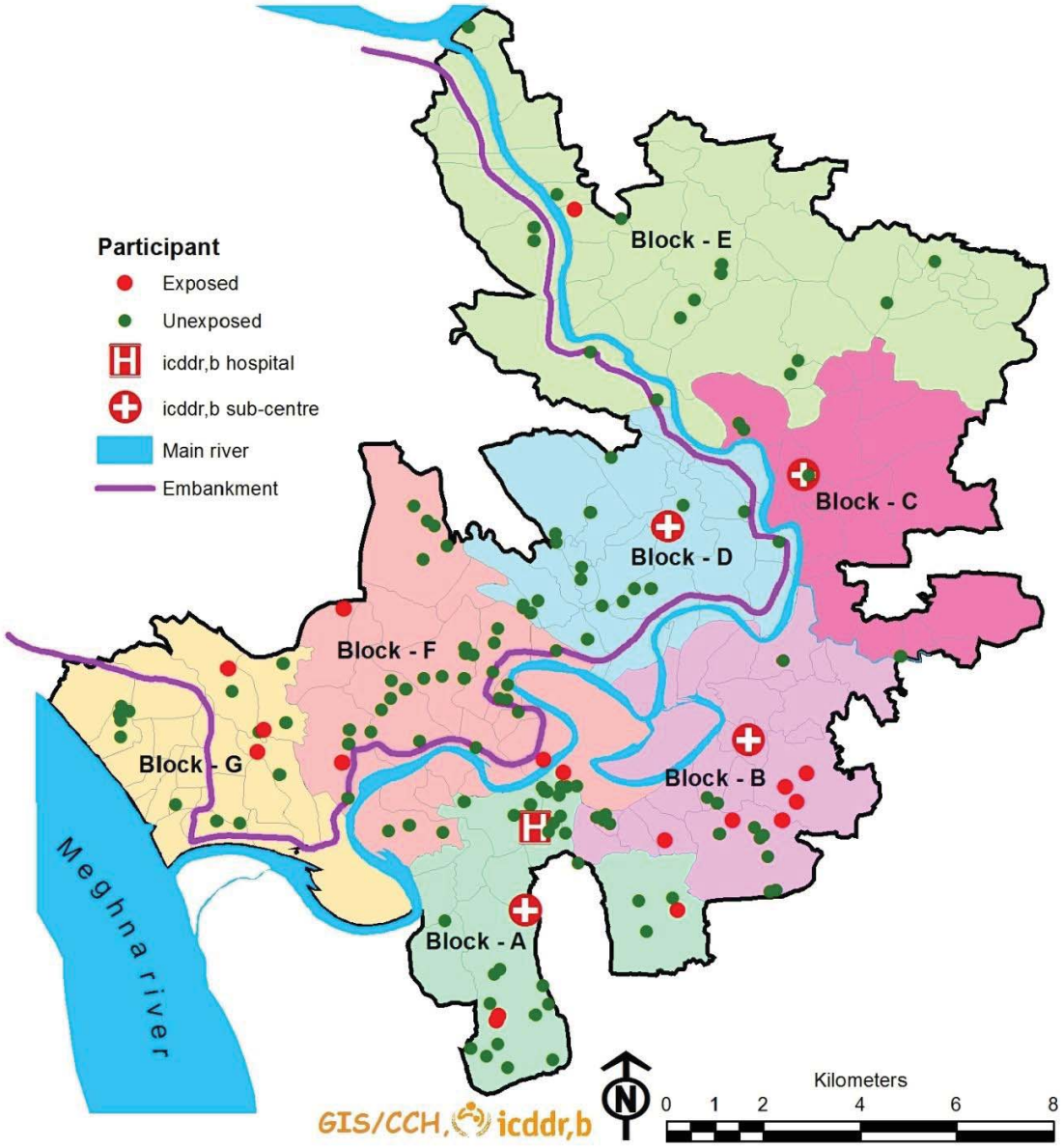

Fig 3: Map showing household locations of exposed and unexposed participants in the study area at Matlab

Anthropometric Measurement

Height and weight was taken for all participants before physical examination and blood pressure measurement. Standing height was measured using a wooden height stick to the nearest of 0.1 cm, and weight was measured using a bathroom scale to the nearest 100gm. Height and weight were both measured twice and the average values recorded.

Examination of skin for arsenic induced lesions

Although arsenic caused skin lesions are uncommon in children age <15 years (1), each child was examined carefully by a trained physician to identify arsenic induced skin changes. Probable and possible skin lesions cases were re-examined by Prof. Allan Smith. Photographs were taken for probable and possible skin lesions cases for review and evaluation.

Biological Sample Collection

Under aseptic precautions, a nurse took 5 ml of venous blood. Blood specimen was kept upright for around 30 minutes and centrifuged to separate serum. Serum was aliquoted into two cryovials and stored at -86°C in Matlab. Samples ware transported to Dhaka with dry ice for further analysis.

A 60 ml urine sample was taken and immediately splitted into 3 aliquots. One was centrifuged to separate epithelial cells. The other two aliquots were stored at -86°C within 1-2 hours.

Sample Size and Power Calculation

This study planned to follow a sub-cohort of 200 participants from the original cohort of 600 children in the study titled “Arsenic and child respiratory health in Bangladesh (ACRH)” (2). To increase study power, we aimed to enrol 100 subjects with the highest exposure and another 100 with the lowest exposure. For respiratory outcomes, the power of the present study was calculated based on the preliminary findings from the ACRH study. In ACRH, the prevalence of respiratory symptoms in non-exposed children ranged from 10% (e.g. child has shortness of breath when walking fast or climbing) to about 30% (e.g. child has woken up with shortness of breath). We believed that there was an ample evidence of lung effects of arsenic to make the

power calculations for 1-tailed tests. For prevalence of respiratory symptoms in the unexposed in the range of 10-20%, þe study power to detect a prevalence odds ratio of 2.5 was in the range of 78% to 89%. Study power for continuous variables like blood pressure was such that we would have 80% power to estimate a difference of 4 units in systolic blood pressure. Estimates of blood pressure standard deviations were taken from a study of children aged 9-16 in India (4).

To obtain the required number of participants, we planned to approach ~110 in each arm considering 10% loss to follow up and non-response.

Data Management and Analysis

Data was entered twice into a personal computer using the Oracle database 10g, cross checked for errors, cleaned and edited. Data was then converted into Microsoft Excel (version Office 2007 for PC) and imported to STATA (version 13) for further analyses. Univariate analysis was done to check the distribution of the key variables. Bivariate analysis using Pearson's chi-square and fisher exact was done to see the independent association. T-test was performed for comparing the means of the continuous variables. Confounders and covariates were selected based on the previously published literature (5-12). A number of studies found sex to significantly modify the impact of arsenic exposure on health outcomes (13-17). Therefore, we considered sex as an effect modifier in our analysis and reported the findings stratified by sex. Multiple regression models were built to determine the independent effects of early life exposure to arsenic through drinking water on respiratory illnesses, blood pressure and renal function.

Ethical Consideration

The institutional review board of the University of California, Berkeley and the International Centre for Diarrhoeal Disease Research, Bangladesh, icddr,b approved the study. For subjects 11 to 17 years, the participants and their parents or guardians were informed in detail about the study objectives and procedures along with its anticipated risks and were asked for assent and consent respectively. Participants and the parents were informed about confidentiality and were assured that irrespective of their participation in this study, they would receive the same medical care from icddr,b. They were informed that they could withdraw from the study at any point even after they agreed to participate in the study. For those aged 18 and older, informed voluntary

consent was taken. They were asked to read the consent form but if they were unable to read, the consent form was read out by our field research assistant (FRA) who asked if they had any queries which were responded to with clear explanations. Again, it was confirmed that participation was totally voluntary and they could withdraw themselves from the study at any time. They were assured about confidentiality. Each study participant was assigned a study identification number in our previous study and it was kept unchanged. Names were kept secret and not entered into the computer.

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Chapter 3

Non-malignant respiratory effects in childhood and adolescence following an early life arsenic exposure through drinking water

Abstract

Background

Several studies have documented increased risk of non-malignant respiratory illnesses including impairment of lung function in adults chronically exposed to arsenic through drinking water. However, there is little evidence on respiratory effects in children and adolescents exposed to arsenic in their early life.

Methods

As described in chapter 2, the study was conducted in rural Bangladesh, Matlab. Participants were previously selected using a population based survey database of 166,934 residents 5 years and above in the sub-district of Matlab. A total of 600 children were selected from this survey, with 110 having early life exposure to arsenic concentrations over 400µg/L and 110 having less than 10µg/L. These two sub-cohorts of children constitute those invited to participate in further follow-up in the current prospective cohort study. Among the exposed and unexposed sub-cohorts, 107 and 93 subjects participated in the study respectively. Histories of respiratory symptoms were collected through a validated structured questionnaire (ISAAC). Lung function (FEV1, FVC) was tested by a portable spirometer following the standard criteria of the American Thoracic Society. Height and weight were measured and socio-demographic data were collected. Water arsenic concentration was measured by hydride generation atomic absorption spectrophotometery.

Results

Chronic respiratory symptoms were evident in male participants from the high arsenic exposure cohort. In an adjusted model, boys with early life arsenic exposure (>400 µg/L) are more likely to report wheezing attacks 1-3 times/week (Prevalence Odds Ratio (POR): 4.99, 95% Confidence Interval (CI): 1.00-24.9, p=0.03), wheezing after exercise (POR: 4.14, 95% CI: 1.05-16.4, p=0.02) and woken up with tightness of chest (POR: 5.01, 95% CI: 1.00-25.0, p=0.02). Significant decreases in FEV1 (-117.3 ml, CI: -246.5, 11.8, p=0.04) and FVC (-135.2 ml, CI: -269.9, -0.37, p=0.02) were observed in male respondents in the high arsenic exposure group compared to the low exposure sub-cohort. Girls from the high exposure sub-cohort did not have respiratory impairment.

Conclusions

High exposure to arsenic in early life has been associated with increased risk of chronic respiratory symptoms and decrements in lung function in male children aged 11-22 years. We plan to follow this unique cohort further to see if the chronic respiratory effects of early life arsenic exposure persist in males as they grow older, and remain absent in females.

Introduction

Arsenic in drinking water has been linked to various non-malignant respiratory illnesses. Several studies in adults have suggested an increased risk of respiratory symptoms and diseases including impairment of lung function following chronic exposure to inorganic arsenic (1-6). Long term exposure to inorganic arsenic has been found to be associated with increased risk of respiratory symptoms like chronic cough, dyspnea and breathlessness and the relationships was dose-dependent (7-10). A cross-sectional study has observed a greater risk of chronic bronchitis with added sounds in chest among people having arsenic induced skin lesion compared to people without lesion (11). Excess mortality from non-malignant lung disease has been reported recently for the same cohort with the highest exposure group having a 75% increased risk of mortality compared to the lowest exposure group (12). A number of studies have observed declines in lung function following exposure to arsenic via drinking water (13-15).

There is growing evidence that early life arsenic exposure has profound health impact in later life. Arsenic readily passes through the placenta (or crosses the placenta) in human beings. Pregnant women exposed to arsenic via drinking water showed similar concentrations in the fetus as in the mother (16). Exposure to arsenic in utero and early childhood results in increased mortality among young adults aged 30-49, from lung cancer and bronchiectasis in Chile. Individuals born during the high arsenic exposure period between 1958 to 1970, the standardized mortality ratios (SMRs) for lung cancer and bronchiectasis were 6.1 (95% CI, 3.5–9.9; $p< 0.001$) and 46.2 (95% CI, 21.1–87.7; $p < 0.001$) respectively (17). A recent finding from Chile revealed increased incidence of lung cancer following moderate arsenic exposure and the association was greater in younger adults exposed to arsenic in early life (18). Lung cancer is considered the major long–term cause of death following exposure to arsenic in drinking water (19, 20). Exposure to arsenic in prenatal life and early childhood via drinking water was found to be associated with impaired lung function. Subjects with early life arsenic exposure (>800 µg/L) had around 12% (P=0.04) lower FEV1 and FVC compared to the lowest exposure group (<50 µg/L). However, the number of in utero exposed subjects was very limited (21).

While there is considerable evidence that early life arsenic exposure increases lung disease in adults, only limited information is available on pulmonary effects in children themselves before adulthood. A study in Antofagasta, Chile in the 1970s reported reduction in prevalence of

respiratory symptoms after supplying arsenic free water (22). In utero and early life exposure to arsenic has also been found to be associated with increased risk of respiratory infections in infants (23, 24). Most studies investigating the impact of arsenic on respiratory health had been performed in exposed adults. Few studies investigated the arsenic exposure during fetal development and early life on respiratory consequences in infants and children. Evidence suggested that early life arsenic exposure resulted in long-term pulmonary consequences that might present much later in life (25) as indicated by increased mortality from bronchiectasis and lung cancer, in adults (17). No study to date has followed the children with well documented early life arsenic exposure to assess long-term health consequences in later part of childhood and as young adults. We are the first to follow a cohort with high arsenic exposure in early life to observe respiratory and other health effects in children, adolescents and young adults (26) (Appendix-7). We now further assess these same children a few years after the first assessment, but confining attention to sub-cohorts with the highest and lowest exposures.

Methods

As described in chapter 2, the study was conducted in rural Bangladesh, Matlab. Participants were previously selected from a population based survey of 166,934 residents of the sub-district of Matlab. A cohort of 300 exposed children aged 7-17 years were selected whose mothers had the highest likely arsenic exposure during pregnancy and another cohort of 300 unexposed children whose mothers' drinking water contained less than 10µg/l. From that cohort, we selected a sub-cohort of 110 children having early life exposure to arsenic concentrations over 400µg/L and another 110 having less than 10 µg/L. These two sub-cohorts of children constitute those invited to participate in further follow-up in the current prospective cohort study. Of them, 107 and 93 children participated in the exposed and unexposed group respectively. Further details of participants' selection and arsenic exposure assessment were described in Chapter-2.

Assessment of Respiratory and other medical illnesses

Detailed histories of respiratory symptoms and disorders including wheezing, asthma, pneumonia, and tuberculosis since our last visit between 2008-10 were taken. Ever and current wheezing i.e wheezing in last 12 months, and asthma was assessed based on the core

questionnaire of the International Study of Asthma and Allergy in Childhood (ISAAC), a valid tool used for the assessment of asthma symptoms in children worldwide (27, 28) . Cough and phlegm, shortness of breath, and family history of asthma was obtained according to an ISAAC supplementary questionnaire (29). A trained physician asked the questions in a structured interview.

Anthropometric and Lung Function Measurement

Height and weight were obtained for all participants before testing lung function. Standing height was measured using a wooden height stick to the nearest of 0.1 cm and weight was measured using a bathroom scale to the nearest of 100gm. Lung function was measured with the EasyOne spirometer (NDD Medical Technologies) which has been used successfully in many studies (13, 21) including our previous one with the same children in Bangladesh (26). It is a handy, portable spirometer that uses ultrasound to measure airflow and the measurement is independent of gas composition, pressure, temperature, and humidity, and eliminates errors due to those variables and made it suitable for the hot and humid conditions in Matlab. After explaining the procedure and demonstrating, subjects were asked to blow out forcibly as hard as possible through the disposable mouthpiece in the standing position without nose clip. The procedure was repeated up to six times in order to produce smooth, reproducible curves that meet American Thoracic Society criteria (30). Spirometry was conducted by the physician who has extensive experience doing spirometry on children. Lung function parameters, primarily FVC (Forced vital capacity), FEV1 (Forced expiratory volume in first second) and FEV1/FVC ratio, were recorded. Test quality is graded from A to F. We achieved grade A in more than 95% cases. In addition, lung function data were reviewed by one of the co-investigators, Prof. John Balmes, pulmonologist at the University of California Berkeley, to ensure quality of the test results.

Ethical Consideration

The institutional review boards of the University of California, Berkeley and icddr,b approved the study. Participants and the parents were informed about the confidentiality and were ensured that irrespective of their participation in this study, they will receive same health care from icddr,b. They were informed that their participation was voluntary, and were also assured they

could withdraw from the study at any time. For subjects 11 to 17 years, parents or guardians along with the participants were informed in detail about the study along with its anticipated risks and were asked for consent and assent respectively. And for those aged 18 and older, informed voluntary consent was taken. They were asked to read the consent form, but if they were unable to read, the consent form was read out by our field research assistant (FRA) who asked if they had any queries which were responded to with clear explanations. Again, it was confirmed that the participation was totally voluntary, and they could withdraw themselves from the study at any time.

Analysis

Univariate analysis was done to evaluate the differences in general characteristics and socio-demographic factors between the low and high exposed cohorts. We performed Fisher's exact test to compare the dichotomous respiratory symptoms between the cohorts separated by sex. Multiple linear regression analyses were used to assess the association of water arsenic concentration with FEV1 and FVC. Firstly, age, sex and height were adjusted. Then more analyses were conducted and adjusted for age, sex, height, weight, mother's education, father's education, father's smoking status and rooms in the house. Logistic regression analysis was conducted to calculate crude and adjusted prevalence odds ratios and 95% confidence intervals for dichotomous respiratory symptoms for the sub-cohort of children exposed to >400 µg/L in their early life and the sub-cohort of children who were not exposed (<10 µg/L). Again, we first adjusted for age and sex, and then conducted further analyses adjusting for age, sex, mother's education, father's education, father's smoking, and number of rooms in the house.

Results

A total of 200 subjects aged 11-22 participated in this study. The mean age was 14.9 (±2.8SD) years. Table 3.1 describes socio-demographic events and characteristics of the study subjects. Among the participants, 107 (53.5%) were exposed to a high arsenic concentrations in early life (>400µg/L) and 93 (46.5%) were exposed to low concentration of As (<10µg/L) in their early life. Of the total 200 subjects, 105 (52.5%) were male and 95 (47.5%) were female. Participants over the age of 17 were more likely to have been exposed to high arsenic concentration (30.84%

vs 16.13%). Both mother and father's education were associated with their children's arsenic exposure. Participants with more educated parents were found to have used lower arsenic concentration water in early life. Fathers of highly exposed children reported more smoking compared to fathers of children in the low exposure group (64.49% vs 50.54%). Respondents residing in higher quality houses were more likely to have had low exposures compared to participants living in mud houses. Other factors like BMI, respondent's educational status, and number of rooms in the household, did not show significant differences between two groups.

Table 3.1: Socio-demographic characteristics of study subjects

Characteristics	Total n (%)	Low Exposure Status<10 µg/l n (%)	High Exposure Status>400µg/l n (%)	P-value
Sex				
Boys	105 (52.50)	44 (47.31)	61 (57.01)	0.171
Girls	95 (47.50)	49 (52.69)	46 (42.99)	
Age (years)				
11-13	72 (36.00)	40 (43.01)	32 (29.91)	0.032
14-16	80 (40.00)	38 (40.86)	42 (39.25)	
17-22	48 (24.00)	15 (16.13)	33 (30.84)	
BMI				
<18.5	145 (72.50)	70 (75.27)	75 (70.09)	0.414
>=18.5	55 (27.50)	23 (24.73)	32 (29.91)	
Education (years)				
No	24 (12.00)	8 (8.60)	16 (14.95)	0.533
1-5	46 (23.00)	23 (24.73)	23 (21.50)	
6-10	117 (58.50)	55 (59.14)	62 (57.94)	
11-13	13 (6.50)	7 (7.53)	6 (5.61)	
No. of family members				
2-4	69 (34.50)	33 (35.48)	36 (33.64)	0.772
5-6	95 (47.50)	46 (49.46)	49 (45.79)	
7-8	22(11.00)	9 (9.68)	13 (12.15)	
9+	14 (7.00)	5 (5.38)	9 (8.41)	
Mother's education				
No education	57 (28.50)	23 (24.73)	34 (31.78)	0.007
Primary	87 (43.50)	34 (36.56)	53 (49.53)	
Secondary and above	56 (28.99)	36 (38.71)	20 (18.69)	
Father's education				
No education	61 (30.50)	25 (26.88)	36 (33.64)	<0.001
Primary	68 (34.00)	18 (19.35)	50 (46.73)	
Secondary and above	71 (35.50)	50 (53.76)	21 (19.63)	
Type of house				
Mud	174 (87.00)	73 (78.49)	101 (94.39)	0.004
Mixed	6 (3.00)	5 (5.38)	1 (0.93)	
Concrete	17 (8.50)	13 (13.98)	4 (3.74)	
Tin	3 (1.50)	2 (2.15)	1 (0.93)	
Rooms in the house				
1	12 (6.00)	6 (6.45)	6 (5.61)	0.954
2	64 (32.00)	28 (30.11)	36 (33.64)	
3	96 (48.00)	46 (49.46)	50 (46.730	
4+	28 (14.00)	13 (13.98)	15 (14.02)	
Father smokes				
Yes	116 (58.00)	47 (50.54)	69 (64.49)	0.046
No	84 (42.00)	46 (49.46)	38 (35.51)	

Prevalence data for respiratory symptoms are presented in Table 3.2. Increases in several respiratory symptoms were found in male subjects in the high exposure group. The main differences were in wheezing attacks (1-3 times/week) (p=0.03), number of nights sleep disturbed (<1/week) (p=0.03), wheezing after exercise (p=0.04), woken up with tightness of chest (p=0.03) and asthma (p=0.04). Female participants in the high exposure group tended to have fewer symptoms than those in the low exposure cohort.

Table 3.2: Prevalence of respiratory symptoms among low and high arsenic exposed children and adolescents

Respiratory Symptoms	Total (n=200) N (%)	Low exposure status(<10µg/L) (n= 93) N (%)	High exposure status(>400 µg/L) (n=107) N (%)	P value*
Coughing				
When having a cold (last 12 months)	131 (65.50)	64 (68.82)	67 (62.62)	0.18
Male	64 (60.95)	26 (59.09)	38 (62.30)	0.45
Female	67 (70.53)	38 (77.55)	29 (63.04)	0.09
When having a cold (last 12 months)	21 (10.50)	64 (68.82)	67 (62.62)	0.50
Male	11 (10.48)	26 (59.09)	38 (62.30)	0.24
Female	10 (70.53)	38 (77.55)	29 (63.04)	0.20
Dry cough (last 12 months)	76 (38.00)	35 (37.63)	41 (38.32)	0.50
Male	42 (40.00)	14 (31.82)	28 (45.90)	0.11
Female	34 (35.79)	21 (42.86)	13 (28.26)	0.10
Wheezing				
Ever	34 (17.00)	15 (16.13)	19 (17.76)	0.43
Male	21 (20.00)	6 (13.64)	15 (24.59)	0.13
Female	13 (13.68)	9 (18.37)	4 (8.70)	0.14
Last 12 months	28 (14.00)	11 (11.83)	17 (15.89)	0.21
Male	17 (16.19)	4 (9.09)	13 (21.31)	0.08
Female	11 (11.58)	7 (14.29)	4 (8.70)	0.30
Number of wheezing attacks (1-3 times)	21 (10.88)	8 (8.89)	13 (12.62)	0.25
Male	13 (12.87)	2 (4.76)	11 (18.64)	0.03
Female	8 (8.70)	6 (12.5)	2 (4.55)	0.14

Number of wheezing attacks (4+ times)	7 (3.91)	3 (3.53)	4 (4.26)	0.50
Male	4 (4.35)	2 (4.76)	2 (4.00)	0.50
Female	3 (3.45)	1 (2.33)	2 (4.55)	0.50
Number of nights sleep disturbed (<1/week)	23 (11.67)	9 (9.78)	14 (13.33)	0.25
Male	12 (11.76)	2 (4.65)	10 (16.95)	0.03
Female	11 (11.58)	7 (14.29)	4 (8.70)	0.26
Number of nights sleep disturbed (1 or more/week)	3 (1.69)	1 (1.19)	2 (2.15)	0.20
Male	3 (3.23)	1 (12.38)	2 (3.92)	0.50
Female	0	0	0	--
Severe enough to affect speech	24 (12.00)	9 (9.68)	15 (14.02)	0.19
Male	15 (14.29)	3 (6.82)	12 (19.67)	0.06
Female	9 (9.47)	6 (12.24)	3 (6.52)	0.28
After exercise	23 (11.5)	7 (7.53)	16 (14.95)	0.06
Male	16 (15.24)	3 (6.82)	13 (21.31)	0.04
Female	7 (7.37)	4 (8.16)	3 (6.52)	0.54
without exercise	7 (3.50)	3 (3.23)	4 (3.74)	0.50
Male	5 (4.76)	1 (2.27)	4 (6.56)	0.30
Female	2 (2.11)	2 (4.08)	0	0.26
When having a cold	28 (14.00)	11 (11.83)	17 (15.89)	0.21
Male	17 (16.19)	4 (9.09)	13 (21.31)	0.08
Female	11 (11.58)	7 (14.29)	4 (8.70)	0.30
When not having a cold	7 (3.50)	2 (2.15)	5 (4.67)	0.23
Male	5 (4.76)	1 (2.27)	4 (6.56)	0.30
Female	2 (2.11)	1 (2.04)	1 (2.17)	0.74
Shortness of breath				
Woken up with shortness of breath	25 (12.50)	10 (10.75)	15 (14.02)	0.26
Male	15 (14.29)	3 (6.82)	12 (19.67)	0.06
Female	10 (10.53)	7 (14.29)	3 (6.52)	0.19
Woken up with tightness of chest	22 (11.00)	8 (8.60)	14 (13.08)	0.18
Male	13 (12.38)	2 (4.55)	11 (18.03)	0.03

Female	9 (9.47)	6 (12.24)	3 (6.52)	0.28
When walking fast or climbing	118 (59.00)	54 (58.06)	64 (59.81)	0.44
Male	58 (55.24)	24 (54.55)	34 (55.74)	0.53
Female	60 (63.16)	30 (61.22)	30 (65.22)	0.43
When walking on level ground	6 (3.00)	0	6 (5.61)	0.02
Male	3 (2.86)	0	3 (4.92)	0.19
Female	3 (3.16)	0	3 (6.52)	0.11
Asthma	28 (14.00)	11 (11.83)	17 (16.04)	0.21
Male	16 (15.24)	3 (6.82)	13 (21.31)	0.04
Female	12 (12.77)	8 (16.33)	4 (8.89)	0.21

Prevalence odds ratios (both crude and adjusted) for respiratory symptoms are shown in table 3.3, 3.3a & 3.3b; first with the sex combined and then separately for males and females. After adjustment for age, father's smoking status and rooms in the house, the prevalence odds ratio (POR) for number of wheezing attacks (1-3 times/week) in the high exposure cohort was increased almost five-fold (POR: 4.99, 95% CI: 1.00-24.9, p=0.03) in male subjects. Other symptoms for highly exposed males were also markedly increased including wheezing after exercise (POR: 4.14, 95% CI: 1.05-16.4, p=0.02) and woken up with tightness of chest (POR: 5.01, 95% CI: 1.00-25.0, p=0.02). Asthma was also found to be increased in exposed males (POR: 4.14, 95% CI: 1.05-16.4, p=0.02). However, increased risks were not observed in females, and in fact most symptoms among females were reduced in the highly exposed cohort compared to the low exposure cohort.

Table 3.4 presents lung function test results analyzed by multiple linear regression analysis. It displays the differences in two major pulmonary function parameters, FEV1 and FVC between the exposed cohort (>400 μg/L) and the unexposed cohort (<10μg/L). We first adjusted for age, sex and height and then more adjustment was done for other potential confounding factors including weight, father's smoking status and rooms in the house. Results showed significant decreases in FEV1 (-117.3 ml, CI: -246.5, 11.8, p=0.04) and FVC (-135.2 ml, CI: -269.9, -0.37, p=0.02) in male participants in the high exposure cohort compared to the low exposure cohort.

Table 3.3: Prevalence odds ratios (PORs) and 95% confidence intervals (CI) for respiratory symptoms comparing low and high arsenic exposure status

Respiratory symptoms		Crude			Adjusted*		
	N	POR	95% CI	p value**	POR	95% CI	p value**
Coughing							
When having a cold (last 12 months)	131	0.76	0.42, 1.37	0.82	0.71	0.38, 1.33	0.86
When not having a cold (last 12 months)	21	0.96	0.39, 2.38	0.53	0.91	0.35, 2.40	0.58
Dry cough (last 12 months)	76	1.03	0.58, 1.83	0.46	0.94	0.51, 1.74	0.58
Wheezing							
Ever	34	1.12	0.53, 2.36	0.38	0.99	0.45, 2.19	0.51
Last 12 months	28	1.41	0.62, 3.18	0.21	1.34	0.56, 3.18	0.25
Number of wheezing attacks (1-3 times)	21	1.48	0.58, 3.75	0.20	1.51	0.57, 4.00	0.21
Number of wheezing attacks (4+ times)	7	1.22	0.26, 5.59	0.40	1.17	0.24, 5.79	0.42
Number of nights sleep disturbed (<1/week)	23	1.42	0.58, 3.45	0.22	1.35	0.53, 3.46	0.27
Number of nights sleep disturbed (1 or more/week)	3	1.82	0.16, 20.5	0.31	1.89	0.15, 23.1	0.31
Severe enough to affect speech	24	1.52	0.66, 3.66	0.17	1.42	056, 3.59	0.23
After exercise	23	2.16	0.85, 5.51	0.05	2.13	0.79, 5.73	0.07
without exercise	7	1.17	0.25, 5.35	0.42	1.38	0.28, 6.84	0.35
When having a cold	28	1.41	0.62, 3.18	0.21	1.34	0.56, 3.18	0.25
When not having a cold	7	2.23	0.42, 11.8	0.17	1.99	0.35, 11.4	0.22
Shortness of breath							
Woken up with shortness of breath	25	1.35	0.58, 3.18	0.24	1.30	0.53, 3.23	0.29
Woken up with tightness of chest	22	1.60	0.64, 4.00	0.16	1.58	0.60, 4.13	0.18
When walking fast or climbing	118	1.08	0.61, 1.89	0.40	0.98	0.53, 1.79	0.47
Asthma	28	1.42	0.63, 3.22	0.20	1.42	0.60, 3.36	0.21

* Adjusted for age, sex, father's smoking status and rooms in the house. Participants' smoking status was not controlled as only two subjects reported history of smoking
** One-tailed *** Two-tailed

Table 3.3a: PORs with 95% CI for respiratory symptoms comparing low and high arsenic exposure status for male

Respiratory symptoms	N	Crude			Adjusted*		
		POR	95% CI	p value**	POR	95% CI	p value**
Coughing							
When having a cold (last 12 months)	64	1.14	0.52, 2.53	0.37	1.03	0.43, 2.45	0.48
When not having a cold (last 12 months)	11	2.06	0.52, 8.27	0.16	1.75	0.39, 7.89	0.23
Dry cough (last 12 months)	42	1.82	0.81, 4.09	0.07	2.04	0.82, 5.06	0.06
Wheezing							
Ever	21	2.07	0.73, 5.84	0.09	1.98	0.66, 5.92	0.11
Last 12 months	17	2.71	0.82, 8.96	0.05	2.97	0.85, 10.4	0.04
Number of wheezing attacks (1-3 times)	13	4.58	0.96, 21.9	0.03	4.99	1.00, 24.9	0.03
Number of wheezing attacks (4+ times)	4	0.83	0.11, 6.18	0.43	0.91	0.11, 7.39	0.54
Number of nights sleep disturbed (<1/week)	12	4.18	0.87, 20.2	0.04	4.31	0.86, 21.8	0.04
Number of nights sleep disturbed (1 or more/week)	3	1.67	0.15, 19.1	0.34	1.99	0.15, 27.1	0.30
Severe enough to affect speech	15	3.35	0.88, 12.7	0.04	3.57	0.90, 14.2	0.04
After exercise	16	3.70	0.99, 13.9	0.03	4.14	1.05, 16.4	0.02
without exercise	5	3.02	0.33, 28.0	0.17	4.48	0.43, 47.1	0.11
When having a cold	17	2.71	0.82, 8.96	0.05	2.97	0.85, 10.4	0.04
When not having a cold	5	3.02	0.33, 28.0	0.17	3.18	0.32, 31.6	0.16
Shortness of breath							
Woken up with shortness of breath	15	3.35	0.88, 12.7	0.04	3.72	0.93, 14.8	0.03
Woken up with tightness of chest	13	4.62	0.97, 22.2	0.03	5.01	1.00, 25.0	0.02
When walking fast or climbing	58	1.05	0.48, 2.29	0.46	0.86	0.37, 2.00	0.64
Asthma	16	3.70	0.99, 13.9	0.03	4.14	1.05, 16.4	0.02

* Adjusted for age, father's smoking status and rooms in the house. Participants' smoking status was not controlled as only two subjects reported history of smoking ** One-tailed

Table 3.3b: PORs with 95% CI for respiratory symptoms comparing low and high arsenic exposure status for female

Respiratory symptoms		Crude			Adjusted*		
	N	POR	95% CI	P value**	POR	95% CI	P value**
Coughing							
When having a cold (last 12 months)	67	0.49	0.20, 1.21	0.94	0.45	0.17, 1.16	0.95
When not having a cold (last 12 months)	10	0.43	0.10, 1.77	0.88	0.48	0.11, 2.09	0.84
Dry cough (last 12 months)	34	0.53	0.22, 1.24	0.93	0.44	0.18, 1.10	0.96
Wheezing							
Ever	13	0.42	0.12, 1.49	0.91	0.32	0.08, 1.24	0.95
Last 12 months	11	0.57	0.16, 2.10	0.8	0.40	0.10, 1.67	0.90
Number of wheezing attacks (1-3 times)	8	0.33	0.06, 1.75	0.91	0.31	0.06, 1.79	0.91
Number of wheezing attacks (4+ times)	3	2.00	0.18, 22.9	0.29	1.30	0.10, 17.5	0.42
Number of nights sleep disturbed (<1/week)	11	0.51	0.16, 2.10	0.80	0.40	0.10, 1.67	0.90
Number of nights sleep disturbed (1 or more/week)	0	--			--		
Severe enough to affect speech	9	0.50	0.12, 2.13	0.83	0.33	0.07, 1.67	0.81
After exercise	7	0.79	0.17, 3.71	0.62	0.64	0.12, 3.40	0.70
without exercise	2	--			--		
When having a cold	11	0.57	0.16, 2.10	0.80	0.40	0.10, 1.67	0.90
When not having a cold	2	1.07	0.07, 17.6	0.48	0.61	0.03, 14.9	0.62
Shortness of breath							
Woken up with shortness of breath	10	0.42	0.10, 1.73	0.89	0.29	0.06, 1.37	0.94
Woken up with tightness of chest	9	0.5	0.12, 2.13	0.83	0.42	0.09, 1.98	0.86
When walking fast or climbing	60	1.19	0.52, 2.74	0.56	1.12	0.46, 2.72	0.41
Asthma	12	0.50	0.14, 1.79	0.86	0.40	0.10, 1.55	0.91

* Adjusted for age, father's smoking status and rooms in the house. Participants' smoking status was not controlled as only two subjects reported history of smoking ** One-tailed

Table 3.4: Results from multivariate linear regression analysis of lung function and arsenic exposure in early life (400+ µg/L compared to less than 10 µg/L)

Lung Function Parameters	Age, sex and Height Adjusted	P value**	More Variables Adjusted*	P value**
FEV1(ml)				
All	-39.8 (-125.4, 45.7)	0.18	-24.4 (-104.50, 55.7)	0.28
Male	-164.2 (-302.9, -25.6)	0.01	-117.3 (-246.5, 11.8)	0.04
Female	100.9 (11.0, 190.8)	0.99	109.2 (27.4, 191.0)	0.99
FVC (ml)				
All	-45.9 (-139.2, 47.4)	0.17	-39.2 (-124.1, 45.6)	0.18
Male	-175.0 (-318.9, -31.1)	0.01	-135.2 (-269.9, -0.37)	0.02
Female	98.4 (-12.4, 209.1)	0.96	91.1 (-2.59, 184.7)	0.97

* Adjusted for age, sex, height, weight, father's smoking status and rooms in the house. Participants' smoking status was not controlled as only two subjects reported history of smoking
** One-tailed

Discussion

To our knowledge, this is the first study that followed a cohort exposed to arsenic in early life to investigate the effects of in utero exposure on respiratory diseases in adolescents and young adults. We observed deleterious the effects of early life arsenic exposure on the respiratory system in boys. After adjustment, boys with in early life arsenic exposure (>400 µg/L) are more likely to report wheezing attacks 1-3 times/week (Prevalence Odds Ratio (POR): 4.99, 95% Confidence Interval (CI): 1.00-24.9, p=0.03), wheezing after exercise (POR: 4.14, 95% CI: 1.05-16.4, p=0.02) and woken up with tightness of chest (POR: 5.01, 95% CI: 1.00-25.0, p=0.02). Marked reduction in FEV1 (-117.3 ml, CI: -246.5, 11.8, p=0.04) and FVC (-135.2 ml, CI: -269.9, -0.37, p=0.02) were observed in male subjects with high arsenic exposure compared to low arsenic exposure. However, these effects are not evident in girls.

Long term exposure to arsenic via drinking water has been implicated with chronic respiratory illnesses in several studies in adults (7-9, 17, 31). A prospective study in Bangladesh has shown an inverse dose–response relationship between arsenic and lung function parameters, FEV1 and FVC. Individuals in the highest baseline water arsenic category (>97 µg/L) has a significant reduction in FEV1 and FVC of 80.6 ml and 97.3 ml respectively. For every one SD increase in water arsenic concentration (118.1 µg/L), FEV1 declined by 46.5 ml and FVC by 53.1 ml (13). A study in India has reported increased respiratory symptoms including impairment in lung

function. Men with arsenic induced skin lesions had a 256.2 ml (95 percent CI: 113.9, 398.4; $p < 0.001$) and 287.8 ml (95 percent CI: 134.9, 440.8; $p < 0.001$) reduction in FEV1 and FVC respectively. For each 100 µg/l increase in arsenic in water FEV1 declines 45.0 ml (95 percent CI: 6.2, 83.9; $p = 0.02$) for FEV1 and FVC 41.4 ml (95 percent CI: -0.7, 83.5; $p =0.054$) in males arsenicosis cases (1). Evidence from Chile suggests that prenatal exposure to arsenic increases the risk of respiratory illnesses later in life (21). A recent study in Mexico has reported significant reduction in forced vital capacity (FVC) in children following exposure to arsenic in utero and early childhood but the association is based on urinary As concentrations which only reflects current arsenic exposure (32).

In our previous study on the whole cohort of 600 children, we found strong associations between respiratory symptoms, especially wheezing when not having a cold (OR=8.41, 95% CI: 1.66-42.6), and shortness of breath when walking fast or climbing (OR=3.19, 95% CI: 1.22-8.32), and prenatal exposure to arsenic more than 500 µg/L (26).

In our original cohort study, we found marked increases in respiratory symptoms in children exposed to inorganic arsenic in their early life but there was little effect on lung function. Increased risk for respiratory symptoms was evident both in boys and girls (26). However, the present study found elevated risk of respiratory symptoms only among males while there was no effect of early life arsenic exposure among females. Interestingly, we observed reduced lung function among the male participants which was absent in the original cohort study. Although these sex specific findings are somewhat surprising, a number of studies have reported gender differences in respiratory effects of arsenic. Pesola et al. found the risk for dyspnea was greater among males compared to females (8). Parvez et, al. observed that arsenic exposure affected FEV1 only in men and the risk for reduced FVC was greater among males (13). Similarly, von Ehrenstein et, al. found increase in respiratory symptoms and marked decline in both FVC and FEV1 in arsenic exposed males, but not in females (1). Dauphine et, al. showed that early life arsenic exposure had a detrimental effect on lung function especially FVC in adult males only (21). Raqib et, al. found that the correlation between prenatal inorganic arsenic and acute respiratory infections was stronger in boys in comparison to girls (24).

A study in Mexico reported that boys living in an arsenic-contaminated area scored poorly on various cognitive tests in comparison to girls living in the same area (33).

Gender variation has also been observed in arsenic induced skin lesions. A number of studies documented increased risk of arsenic induced skin lesions in males compared to females (34-37). This may be due to sex differences in arsenic metabolism. Males are reported to have reduced methylation capacity than females so more is present in inorganic form, or as MMA3, both of which are highly toxic (38, 39). Although one might expect stronger finding in males than in females, we have no explanation for this sex specific findings in this follow-up compared to our first study. It seems that the respiratory symptom effects in girls have completely disappeared, while boys continue to have symptoms and now also have evidence of reduced lung function. Further follow-up of these children, including urinary arsenic speciation, may help elucidate these puzzling observations.

The mechanisms by which arsenic causes chronic respiratory illness via drinking water are not well understood. Immune suppression is considered one of the possible ways. A review of evidence suggested that arsenic causes immune suppression by inducing apoptosis (40, 41), oxidative stress (42, 43) and inflammation (43, 44). Early life arsenic exposure through drinking water has found to be associated with reduced size and function of thymus leading to immune suppression and increase susceptibility to infection (24, 45). Chronic exposure to arsenic through drinking water alters respiratory epithelial barrier and impairs wound repair through the upregulation of MMP-9 by pulmonary epithelial cells (46).

One of the strengths of the current study is the individual meticulous assessment of drinking water arsenic exposure level since prenatal life including change of exposure over the time. A limitation of the study was that we did not take the arsenic concentration of any tube well sources that was used for less than one month; which might affect exposure status to some extent.

Conclusion

Our study suggests that the exposure to high concentration of arsenic in early life increases the risk of chronic respiratory symptoms including decline in lung function in male children. We plan to follow the full unique cohort of 600 children further to see if the chronic respiratory effects of early life arsenic exposure persist in males as they grow older, and remain absent in females. The public health impact of early life arsenic exposure could be substantial as many

children in Bangladesh had exposure to arsenic in early life and many are currently still drinking arsenic contaminated water. Safe water is an urgent requirement to avoid arsenic related health consequences.

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Chapter 4

Association of early life arsenic exposure with prehypertension in children and adolescents in rural Bangladesh

Abstract

Background

Chronic exposure to arsenic through drinking water has been implicated with elevated blood pressure in adults. However, few studies have evaluated the impact of early life arsenic exposure on blood pressure in children, and this is the first study assessing older children and adolescents, aged 11 to 22 years when blood pressure was measured.

Methods

The study is conducted in Matlab in rural Bangladesh. A subcohort of 107 participants with high early life arsenic exposure (>400 µg/L) and another subcohort of 93 subjects with low early life exposure (<10 µg/L) were selected from a cohort of 600 children who had their lifetime data on arsenic concentration in drinking water, obtained by us in an earlier study. Blood pressure has been recorded using a standard mercury sphygmomanometer with an appropriately sized blood pressure cuff. Prehypertension in children was defined as systolic blood pressure (SBP) and/or diastolic blood pressure (DBP) between the 90th and 95th percentile for age, sex and, height. For the subjects 18 years and above, prehypertension was classified as SBP above 120 and ranged from 120-139 mmHg, and/or DBP above 80 (range 80-89 mmHg).

Results

The overall prevalence of prehypertension was 16.5%. The high exposure subcohort had a higher prevalence of prehypertension compared to the low exposure group (20.6% vs 11.8%, 1-tailed p = 0.070,). After adjustment for potential confounders, the prevalence odds ratio (POR) for pre-hypertension was 1.68 [95% Confidence Interval (CI): 0.65-4.35, p=0.14] in children and adolescents exposed to arsenic >400 µg/L in their early life. The increased prevalence of

prehypertension was only evident in females (POR=3.93, 95% CI: 0.79-19.6, p=0.05), not in boys (POR= 0.85, 95% CI: 0.23-3.15, p=0.60).

Conclusions

Our results suggest a possible association between early life arsenic exposure and increased risk of prehypertension in girls, but not in boys. This sex differences was not expected, and the findings need to be confirmed.

Introduction

Arsenic, a ubiquitous element naturally present in groundwater, poses a major public health threat globally. Several studies suggest that long term exposure to inorganic arsenic through drinking water increases the risk of cardiovascular disorders including hypertension (1-7). Studies in Taiwan and Bangladesh have documented that people chronically exposed to high levels of arsenic through drinking water have a higher prevalence of hypertension, and the associations were dose dependent (8, 9). A recent study in Taiwan also reported a dose-response relationship between urinary arsenic and hypertension (10). Elevated blood pressure has been observed among women of child bearing age; exposed to arsenic via drinking water in Inner Mongolia, China. Mean systolic and diastolic pressure rose 6.83 mm Hg (95% CI 5.39, 8.27) and 3.08 mm Hg (95% CI 1.84, 4.31), respectively in individuals with the highest quartile of water arsenic concentration (>100 μg/L) compared to lowest quartile (<20 μg/L) (11). Another recent study from the same place has reported an association between long-term arsenic exposure and increased risk of hypertension. The prevalence of hypertension was raised nearly 2-fold in the high exposure group (>50 μg/L) compared to low the low exposure group (<10 μg/L) and the association is dose dependent (12). Elevation of pulse pressure has been found to be associated with long term arsenic exposure in drinking water in Bangladesh and the relationship was dose dependent (13). Evidence from a meta-analysis of several epidemiological studies in different countries shows an association between long term exposure to high concentration of arsenic and cardiovascular disease (14).

There is growing evidence that early life exposure to environmental toxicants increase the risk of developing a wide range of chronic diseases in adults. Exposure to high levels of arsenic in utero and/or early childhood has been implicated with increased risk of morbidity and mortality from various malignant and non-malignant illnesses in adults (15-18). Young adult males aged 30–49 years who were exposed to arsenic in prenatal and early life had more than three times increased rate of mortality from acute myocardial infarction (19). A recent study in Bangladesh has reported a modest increase in blood pressure among the children aged 4.5 years with pre and postnatal exposure to arsenic through drinking water (20). Several studies documented the association between arsenic and elevated blood pressure in adults (6, 13, 21) but so far we know, this is the only study that evaluated the impact of early life arsenic exposure on blood pressure in

children (20). No studies look at the effects of early life arsenic exposure on blood pressure in older children and adolescents. Thus we planned to investigate the effects of early life arsenic exposure on blood pressure among children and adolescents with detailed and individual arsenic exposure level in drinking water.

Methods

The study was conducted in Matlab, a sub-district around 55 km southeast of the capital city, Dhaka. An exposed sub-cohort of 110 children with early life arsenic exposure ((>400 μg/L) and another unexposed sub-cohort of 110 children with low exposure (<10 μg/L) were selected from a cohort of 600 children having lifetime arsenic concentration status. Of the two sub-cohorts, 107 exposed and 93 unexposed subjects participated in this follow up study. Details of participants' selection and arsenic exposure assessment were described in chapter two.

Anthropometric and Blood Pressure Measurement

Height and weight was taken for all participants before blood pressure measurement. Standing height was taken using a locally made wooden height stick to the nearest 0.1 cm and weight was measured using a bathroom scale to the nearest 100gm. Blood pressure was measured by a trained physician using a standard mercury sphygmomanometer in sitting position. Paediatric cuffs with appropriate size and a standard adult cuff were used for children and young adults respectively. Systolic blood pressure was defined by the onset of the first Korotkoff sound (K1) and diastolic blood pressure was marked as the disappearance of Korotkoff sounds or fifth Korotkoff sound (K5, the last sound heard). Two readings were taken at 5 minutes interval and the average value was taken as the participant's blood pressure (22). For the participants age below 18 years, blood pressure was converted into percentile based on age, height and sex and prehypertension was defined either SBP (systolic blood pressure) and/or DBP (diastolic blood pressure) fell between 90^{th} to 95^{th} percentile or blood pressure was ≥120/80 mmHg irrespective of percentiles. Hypertension was defined as average SBP and/or DBP that was greater than or equal to 95th percentile for sex, age, and height (23). For subjects aged 18 years and above, prehypertension was defined if SBP fell between 120-139 mmHg and/or DBP 80-89 mmHg and participants were categorized as hypertensive if they had SBP ≥140 mmHg and/or DBP ≥90

mmHg (24). We got only two hypertensive cases and as the number was very few, we included them in the prehypertension group for further analysis.

Ethical Consideration

The study was approved by the institutional review board of the University of California, Berkeley and icddr,b. Informed written voluntary consent was taken from the participants 18 years and above. For the younger subjects both assent of the participants and consent from a parent or guardian was taken. Participants and parents were explained regarding the objectives of the study, risk/benefits, confidentiality and rights of withdrawing from the study even after giving consent.

Analysis

To compare the general characteristics and socio-demographic characteristics between the low and high exposure group, univariate analysis was done using chi-square and Fisher's exact test. Mean differences in systolic, diastolic and pulse pressure between the two groups was assessed by student's t-test. A multiple logistic regression was conducted to estimate the odds ratio with 95% confidence interval for prehypertension. We first adjusted for age, sex and BMI and then father's smoking, and number of rooms in the house was included in the model. Age and BMI were inserted as continuous variables.

Results

Of the total 200 cohort subjects enrolled in the study, 105 (52.5%) were male and rest 95 (47.5%) were female. The mean age of the participants was 14.9 (±2.8) years. General characteristics and socio-demographic events of the study subjects by exposure are presented in table 4.1. Among the participants, 107 (53.5%) were in the high arsenic (>400µg/L) exposure group and 93 (46.5%) were in the low (<10µg/L) exposure group. Variables like sex, respondent's educational status, number of rooms in the household did not show any significant differences between two groups. Children with high Body mass index (BMI) were more frequent in the high exposure group (51.5% vs 48.5%). Participants having parents with secondary and higher degrees were found to fall more into low exposure category (38.7% and 53.8% Vs 18.7%

and 19.6% maternal and paternal education respectively). Dwellers of better housing condition i.e. other than mud were found more in lower exposed group (21.5% Vs 5.6%).

Table 4.1: Socio-demographic characteristics of study subjects

Characteristics	Total n (%)	Low Exposure Status<10 µg/l n (%)	High Exposure Status>400µg/l n (%)	P-value
Sex				
Boys	105 (52.50)	44 (47.31)	61 (57.01)	0.171
Girls	95 (47.50)	49 (52.69)	46 (42.99)	
Age (years)				
11-13	72 (36.00)	40 (43.01)	32 (29.91)	0.032
14-16	80 (40.00)	38 (40.86)	42 (39.25)	
17-22	48 (24.00)	15 (16.13)	33 (30.84)	
BMI				
<18.5	145 (72.50)	70 (75.27)	75 (70.09)	0.414
>=18.5	55 (27.50)	23 (24.73)	32 (29.91)	
Education (years)				
No	24 (12.00)	8 (8.60)	16 (14.95)	0.533
1-5	46 (23.00)	23 (24.73)	23 (21.50)	
6-10	117 (58.50)	55 (59.14)	62 (57.94)	
11-13	13 (6.50)	7 (7.53)	6 (5.61)	
No. of family members				
2-4	69 (34.50)	33 (35.48)	36 (33.64)	0.772
5-6	95 (47.50)	46 (49.46)	49 (45.79)	
7-8	22(11.00)	9 (9.68)	13 (12.15)	
9+	14 (7.00)	5 (5.38)	9 (8.41)	
Mother's education				
No education	57 (28.50)	23 (24.73)	34 (31.78)	0.007
Primary	87 (43.50)	34 (36.56)	53 (49.53)	
Secondary and above	56 (28.99)	36 (38.71)	20 (18.69)	
Father's education				
No education	61 (30.50)	25 (26.88)	36 (33.64)	<0.001
Primary	68 (34.00)	18 (19.35)	50 (46.73)	
Secondary and above	71 (35.50)	50 (53.76)	21 (19.63)	
Type of house				
Mud	174 (87.00)	73 (78.49)	101 (94.39)	0.004
Mixed	6 (3.00)	5 (5.38)	1 (0.93)	
Concrete	17 (8.50)	13 (13.98)	4 (3.74)	
Tin	3 (1.50)	2 (2.15)	1 (0.93)	
Rooms in the house				
1	12 (6.00)	6 (6.45)	6 (5.61)	0.954
2	64 (32.00)	28 (30.11)	36 (33.64)	
3	96 (48.00)	46 (49.46)	50 (46.730	
4+	28 (14.00)	13 (13.98)	15 (14.02)	
Father smokes				
Yes	116 (58.00)	47 (50.54)	69 (64.49)	0.046
No	84 (42.00)	46 (49.46)	38 (35.51)	
Blood Pressure Status				
Normal	167 (83.5)	82 (88.17)	85 (79.44)	0.070*
Prehypertension	33 (16.5)	11(11.83)	22 (20.56)	

*1-tailed P value obtained by Fisher's exact test

Overall prehypertension prevalence was 16.5%; Children and adolescents in the high exposure group had a higher prevalence (20.6%) compared to those in the low exposure sub-cohort (11.8%); 1-tailed p=0.070.

Mean systolic and diastolic pressure were slightly higher in the high exposure group compared to the low exposure sub-cohort (103.0±11.9 vs. 101.7±9.9 and 64.2±7.4 vs. 63.04±5.07 respectively (95% CI: -4.3-1.8; p=0.42) (Table 4.2). The difference is more pronounced in female subjects. Mean systolic blood pressure in females was 101.4±9.4 mm Hg and 99.7±9.2 mmHg (95% CI: -5.5-2.1; p=0.37) and mean diastolic blood pressure was 64.2±6.8 mmHg and 62.5±4.7 mmHg (95% CI: -4.1-0.7; p=0.17) in the high and low exposure groups respectively. In contrast, male respondents did not show differences in mean systolic blood pressure 104.1±13.5 mm Hg and 104.0±10.4 mmHg (95% CI: -5.0-4.7; p=1.0) and mean diastolic blood pressure was 64.2±7.9 mmHg and 63.6±5.4 mmHg (95% CI: -3.2-2.0; p=0.65) between the high and low exposure groups.

Table 4.2: Mean SBP, DBP and Pulse pressure between low and high level of As exposure group

Characteristics	Low Exposure Status<10 µg/l; Mean (SD)	High Exposure Status>400µg/l ; Mean (SD)	p- value (95% CI for mean difference)
SBP	101.72(9.93)	102.96 (11.89)	0.42 (-4.28, 1.80)
Male	104.0 (10.38)	104.1 (13.45)	0.95 (-4.96, 4.66)
Female	99.69 (9.15)	101.4 (9.35)	0.37 (-5.49, 2.05)
DBP	63.04(5.07)	64.21(7.41)	0.19 (-2.92, 0.59)
Male	63.64 (5.43)	64.23 (7.92)	0.65 (-3.18, 1.99)
Female	62.51 (4.72)	64.17 (6.75)	0.17 (-4.06, 0.73)
Pulse Pressure	38.68(8.52)	38.76(9.55)	0.95 (-2.62, 2.46)
Male	40.34 (8.72)	39.90 (10.17)	0.82 (-3.32, 4.20)
Female	37.18 (8.13)	37.24 (8.52)	0.97 (-3.45, 3.34)

Multiple logistic regression (Table 4.3) was done between prehypertension and arsenic exposure status. The prevalence odds ratio (POR) for prehypertension was 1.44 (95% CI: 0.6-3.6; p=0.21) in the high arsenic exposure group compared to the low exposure when adjusted for age, sex and BMI. It rose to 1.68 (95% CI: 0.7-4.4; p=0.14) when more variables like fathers' smoking status and number of rooms in the house were adjusted. When stratified by sex, the effect was much more pronounced in females with nearly a 4-fold increased risk of developing pre-hypertension (POR=3.93, 95% CI: 0.8-19.6, p=0.05), whereas in males it was not increased at all (POR=0.85, 95% CI: 0.2-3.2, p=0.60).

Table 4.3: Results from multiple logistic regression analysis of prehypertension* and early life arsenic exposure status (400+ µg/L compared to less than 10 µg/L)**

Blood Pressure Status	Age, Sex and BMI Adjusted POR(95% CI)	P value**	More Variables Adjusted* POR(95% CI)	P value**
Prehypertension				
All	1.44 (0.58, 3.58)	0.21	1.68 (0.65, 4.35)	0.14
Male	0.72 (0.21, 2.52)	0.70	0.85 (0.23, 3.15)	0.60
Female	3.24 (0.71, 14.78)	0.06	3.93 (0.79, 19.6)	0.05

* Adjusted for age, sex, BMI, father's smoking status and rooms in the house
** One-tailed
*** Prehypertension was defined earlier in method section under blood pressure measurement

Discussion

This is the first study that investigated the effects of early life arsenic exposure on blood pressure in children and adolescents. We found an association between early life exposure to high concentration of arsenic in drinking water and elevated blood pressure classified as prehypertension in females only. Children and adolescents with early life exposure to arsenic more than 400 µg/L were 1.68 times at risk of developing prehypertension (POR=1.68 (95% CI: 0.6-3.6; p=0.21). However, the effect was more pronounced in girls who had a nearly four-fold increased risk of having prehypertension.

No studies so far reported sex specific blood pressure effects in individuals exposed to arsenic through drinking water. However, a few studies reported that long term arsenic exposure increases the risk of cardiovascular disorders in females (25, 26). Chen et, al. found that chronic exposure to arsenic through drinking water increased the risk of developing QT-interval prolongation in women. Adjusted Odds Ratios (ORs) of QT-interval prolongation in women was 1.24 (95% CI: 1.05, 1.47) and 1.24 (95% CI: 1.01, 1.53) for a 1-SD increase in baseline drinking water and urinary arsenic, respectively (25). Similarly, Mumford et.al. reported susceptibility of women to QT interval prolongation compared to men when chronically exposed to arsenic (26).

Chronic exposure to arsenic via drinking water has been associated with increased risk of elevated blood pressure in adults in different countries including Bangladesh (6, 9, 10, 27). A systematic review found a pooled odds ratio for hypertension of 1.27 (95% CI: 1.09, 1.47) in exposed individuals with average arsenic concentration ≥50µg/L (28). A recent longitudinal study in Bangladesh has reported a link between arsenic exposure from drinking water and changes in blood pressure over the years. Individuals with the highest concentration of water arsenic at baseline had 0.48 and 0.39 mmHg greater annual increase for systolic and diastolic blood pressure respectively (29). A cohort study in Bangladesh found that children exposed to arsenic during their prenatal life showed a mild increase in blood pressure at the age of 4.5 years. Arsenic exposure was measured by arsenic concentration in mother's urine and 1 mg/L increase in maternal urinary arsenic was associated with 3.7 mm Hg and 2.9 mm Hg increase in mean systolic and diastolic pressure respectively (20). Another recent study among children 3-8 years old in Mexico also found an association between early arsenic exposure and elevated blood pressure in childhood. For each 1-µg/mL increase in total arsenic measured in urine (U-tAs) was

associated with 21mmHg (95% CI: 4, 37; p=0.015) increase in systolic blood pressure and 13 mmHg (95% CI: 2, 24, p=0.023) in diastolic blood pressure (30).

The mechanism of arsenic induced hypertension is not well established. However, several epidemiological studies suggested that arsenic in drinking water increases the risk of various cardiovascular disorders (2, 5, 25, 31, 32). Early life exposure to arsenic has also been reported to increase mortality from myocardial infarction in young adults in Chile (19). An ecological study in an arsenic endemic village in south-western Taiwan found a dose–response relationship between high levels of arsenic exposure (700–930 μg/L) and carotid atherosclerosis, defined based on carotid artery intima-media thickness and carotid plaque (33). Increased risk of carotid atherosclerosis has been observed in north-eastern Taiwan with low to moderate arsenic exposure and the adjusted prevalence odds ratio (POR) for carotid atherosclerosis was 2.6 (95 % confidence interval [CI] 1.3–5.0) among the highest exposure group (>100 μg/L) compared to the lowest (≤50 μg/L) (34).

Endothelial dysfunction is considered to be one of the most convincing mechanisms in arsenic induced cardiovascular diseases including hypertension (35). Chronic inflammation is considered another mechanism for developing cardiovascular disorders and exposure to arsenic has been found to be associated with chronic vascular inflammation (36). Arsenic induced oxidant stress and increase production of reactive oxygen species (ROS) might also have a role in cardiovascular toxicity (37, 38). Inorganic arsenic has been found to be associated with increased plasma low density lipoprotein (LDL) and C-reactive protein (CRP) (39). Arsenic impairs endothelial function by increasing calcium influx in the cell (40). Prenatal exposure to arsenic induces atherosclerotic changes in ApoE knockout mice and postnatal and exposure accelerates atherosclerosis, oxidative stress and vascular inflammation (41, 42).

One of the strengths of the current study is the individual assessment of drinking water arsenic exposure levels since early life including change in exposure over time.

A limitation of the study is absence of information of intake of arsenic from food and other sources. However with the arsenic water concentrations in this study, drinking water is the overwhelming source of arsenic exposure.

Conclusion

Our study found that early life arsenic exposure increased the risk of developing prehypertension in female children and adolescents. Since millions of people including pregnant women are drinking arsenic contaminated water, a modest risk of prehypertension could have substantial long term health effects. Exposure needs to be prevented anyway, and exposure in early life should be a high public health priority.

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Chapter 5

Effects of early life arsenic exposure on renal function in children and adolescents in rural Bangladesh

Abstract

Background

Long term exposure to elevated arsenic in drinking water has been reported to increase the risk of chronic kidney disease in adults. However, information on renal effects of arsenic in children and adolescents exposed to inorganic arsenic in their early life is very limited. We therefore assessed renal function in children and adolescents with early life arsenic exposure in rural Bangladesh, in what we believe is the first study involving older children over age 11 and adolescents.

Methods

As described in chapter 2, the study was done in Matlab, a sub-district of Bangladesh. Participants were selected from a previously conducted population based survey of 166,934 residents in Matlab. A total of 600 children aged 7-17 years were selected from this survey, with 110 subjects having early life arsenic exposure over 400µg/L and another 110 having less than 10µg/L. These two sub-cohorts of children were invited to participate in further follow-up in the current prospective cohort study. Of them, 107 exposed and 93 unexposed subjects agreed and enrolled in this study. Serum beta- 2-microglobulin (β2MG), considered a marker of early renal injury, was determined by the Immunoturbidimetric assay. Participants were categorized having normal or elevated β2MG based on the age and sex specific normal reference values. The estimated Glomerular Filtration Rates (eGFR) were calculated from serum β2MG for easy interpretation of renal function.

Results

Participants with high early life arsenic exposure (>400µg/L) had nearly 2-fold higher risk of elevated β2MG compared to those exposed to low (<10 µg/L) [odds ratio (OR) =1.98; 95% Confidence interval (CI): 1.12-3.49; p=0.010] in an adjusted model. The effect was more pronounced in girls (OR=3.02, 95% CI: 1.29-7.10; p=0.006) with little evidence in boys (OR=1.36, 95% CI: 0.62-3.00; p=0.222). Likewise, the odds ratio (OR) for reduced eGFR (<95 mL/min/1.73 m2) increased nearly 2-fold in exposed children [OR=1.81; 95% Confidence Interval (CI): 0.97 to 3.37, p=0.031] after adjustment. The effect was largely confined to females (OR=2.89; 95% CI: 1.13, 7.38, p=0.013) with little evidence in males (OR=1.29; 95% CI: 0.53, 3.14, p=0.288).

Conclusions

We found evidence of reduced kidney function as indicated by elevated serum β2MG and reduced eGFR and the effect was more pronounced in female children and adolescents who had been exposed to high arsenic concentrations in their early life. We plan to follow all these children to assess the progression of changes as they become older.

Introduction

Long term exposure to arsenic via drinking water is a major public health concern worldwide. Millions of people throughout the world particularly in Bangladesh, West Bengal, Taiwan, Nepal, and Mongolia are chronically exposed to arsenic-contaminated drinking water (1). Prolonged exposure to high concentrations of arsenic has been found to be associated with increased risk of malignant and non-malignant renal diseases (2-6). Studies in high arsenic endemic areas in southwestern Taiwan have reported increased risk of death from renal cancer and other kidney diseases (7, 8). Mortality from malignant and non-malignant kidney diseases tended to decline after installation of new water supplies and reduction of arsenic concentrations in drinking water; supporting a causal relationship between chronic exposure to arsenic and chronic renal disease (9, 10). A recent study in Northern Chile has reported an elevated risk of cancers of the kidney and ureter following long term exposure to high levels of arsenic in drinking water, and the association was dose dependent (11). An increased prevalence of renal diseases has been observed in arsenic endemic areas in Taiwan. After controlling for age and sex, there was nearly 3-fold increase in renal diseases among the diabetic and 1.5 times increase in non-diabetic people in endemic area compared to diabetic and non-diabetic residents in non-endemic area (12).

Chronic exposure to elevated levels of arsenic in drinking water has been linked to impaired renal function. A study in an adult population in Bangladesh with wide range of exposure to arsenic from drinking water has reported a dose response relationship between concentration of arsenic in well water and the prevalence of proteinuria (13). Elevated urinary beta2 microglobulin has been observed in a population co-exposed to arsenic and cadmium in China (14). A recent review has documented a positive association between arsenic and chronic kidney diseases which was more evident in populations exposed to high levels of arsenic in drinking water (15).

Emerging evidence from Chile suggests that early life exposure to arsenic through drinking water increases risks of both malignant and non-malignant renal disease in adulthood. In Antofagasta, Chile, mortality from chronic renal diseases including renal failure was increased around 2-fold [SMR=2.0; 95% confidence Interval (CI): 1.5 to 2.8] in young adults, born just before or during the high-exposure period of 1958–1970 (16). A recent study in Chile has

reported a 7-fold rise in death from kidney cancer [SMR=7.1; 95% Confidence Interval (CI): 3.1 to 14] in young adults aged 30-39 years exposed to high concentration of arsenic in the prenatal period and/or early childhood (17). However, few studies have investigated the effects of early life arsenic exposure on kidney function in children and adolescents and the findings are inconsistent. A prospective study in Bangladesh has reported mild impairment in renal function, measured by estimated glomerular filtration rate (eGFR) in children 4.4-5.4 years old. Urinary arsenic in utero at 8 weeks of gestation and during infancy was negatively associated with (eGFR) (18). However, a recent paper on the same cohort of children but using cross-sectional data on outcome and exposure did not observe a relationship of arsenic in urine with renal function (19). Here we present our findings in older children and adolescents assessing those who had been exposed to high water arsenic concentrations in early life.

Methods

As described in chapter 2, the study was conducted in rural Bangladesh, Matlab around 55 km southeast of the capital city, Dhaka. Participants were previously selected from a population based survey of all 166,934 residents in sub-district of Matlab. A total of 600 children were selected from this survey, with 107 having early life exposure to arsenic concentrations over 400µg/L and 93 having less than 10 µg/L arsenic exposure in early life. These two sub-cohorts of children were enrolled in further follow-up in the current prospective cohort study. Further details of participant selection and arsenic exposure assessment have been described in chapter two. To assess renal function impairment at the early stage we measured serum beta-2 microglobulin (β2MG), which is considered as one of the most sensitive biomarkers in detecting early renal toxicity in occupational or environmental exposure to arsenic and other heavy metals (14, 20). Blood samples in our study were collected aseptically and processed and stored at -86º C and transported on dry ice to Dhaka for analysis. β2-microglobulin was determined by the Immunoturbidimetric assay using Roche automated clinical chemistry analyzers Hitachi-902 (Roche, Germany). The lower detection limit of the test was 0.03 mg/L and the measurement range was (0.2-5.80) mg/L (21). Participants were categorized into normal and elevated β2MG groups based on individual age and sex specific normal reference values of serum β-2 microglobulin from a study on Japanese children aged 1-16 years, (Table 5.1) (22). As we didn't

find age and sex specific reference values for the participants aged 17-22 years, we used the cut off value for16 years.

Table 5.1: Median, 2.5th percentile, and 97.5th percentile of serum β2MG reference value in each age group according to sex

Age	All subjects				Boys				Girls			
	n	**2.5%**	**50%**	**97.5%**	***n***	**2.5%**	**50%**	**97.5%**	***n***	**2.5%**	**50%**	**97.5%**
3-5 months	21	1.5	1.8[a]	3.2	17	1.5	1.8	3.2	4	1.6	1.8	2.1
6-8 months	18	1.4	1.8[a]	2.6	14	1.4	1.9	2.6	4	1.6	1.6	2.3
9-11 months	29	1.3	1.7[a]	3.3	15	1.3	1.7	3.3	14	1.3	1.8	3.2
1 year	69	1.4	1.7[a]	3.1	32	1.4	1.7	3.2	37	1.2	1.6	3.0
2 years	73	1.0	1.5	2.5	40	1.0	1.5	2.2	33	1.0	1.5	3.4
3 years	85	1.0	1.5	2.3	46	1.1	1.5	2.3	39	1.0	1.5	2.4
4 years	78	1.1	1.4	2.5	42	1.0	1.4	2.1	36	1.1	1.4	3.1
5 years	94	1.1	1.4	2.3	46	1.1	1.5	2.7	48	1.0	1.4	2.2
6 years	101	1.1	1.4	2.3	43	1.1	1.4	2.4	58	1.0	1.5	2.3
7 years	83	1.0	1.4	2.1	36	0.9	1.3	2.1	47	1.0	1.4	2.2
8 years	55	1.0	1.4	2.5	19	1.0	1.4	1.8	36	1.0	1.4	2.3
9 years	37	1.0	1.4	2.1	18	1.1	1.4	1.8	19	1.0	1.4	2.1
10 years	42	0.9	1.3	1.9	11	1.1	1.4	1.6	31	0.9	1.3	1.9
11 years	58	1.0	1.3	2.3	19	1.1	1.3	2.1	39	1.0	1.2	2.4
12 years	69	1.0	1.3	1.8	14	1.2	1.3	1.5	55	0.9	1.3	1.9
13 years	68	1.0	1.3	1.8	30	1.0	1.4	2.0	38	1.0	1.2	1.5
14 years	57	0.9	1.3	2.0	17	1.1	1.4	2.0	40	0.9	1.2	1.7
15 years	35	0.8	1.2	1.8	15	0.8	1.2	1.8	20	0.8	1.1	1.7
16 years	59	0.8	1.2	1.8	30	0.8	1.2	1.8	29	0.8	1.1	1.4
All ages	1311	1.0	1.4	2.3	504	1.0	1.4	2.3	627	1.0	1.4	2.3

* $p < .0001$ in comparison to the mean value in all subjects

[Reproduced from Ikezumi et, al. 2013]

Estimated glomerular filtration rate (eGFR) was calculated by using the formula (eGFR = 149*(1/serum β2 microglobulin in mg/L) + 9.153) and categorized into two; normal (≥95 mL/min/1.73 m2) and reduced (<95 mL/min/1.73 m2) based on a recent study in Japan (23). GFR is commonly considered the best index of overall kidney function and may be useful in assessing any decline in renal function at early stage (24, 25).

Ethical Consideration

The institutional review board of the University of California, Berkeley and icddr,b approved the study. Participants and their parents were informed about confidentiality and were assured that, irrespective of their participation in this study, they would receive same health care from icddr,b. They were informed that their participation was voluntary and they were also assured that they could withdraw from the study at any time. For subjects 11 to 17 years of age, parents or guardians along with the participants were informed about the study along with its anticipated risks and were asked for consent and assent respectively. For older subjects aged over 18 years, voluntary informed written consent was taken explaining the risk/benefits, confidentiality and right of withdrawing from the study at any point.

Statistical Analysis

Data were analyzed using STATA 13 (Stata Corporation, College Station, TX, USA). We performed Fisher's exact test to compare the dichotomous kidney function parameters (β2MG and eGFR) between the high and low arsenic exposure groups separated by sex. Multiple logistic regression analysis of β2MG and eGFR stratified for sex was done to assess the effect of arsenic exposure on renal function.

Results

In total, 200 participants aged 11 to 22 years participated in the study. Their mean age was 14.9 (±2.8) years. Socio-demographic characteristics of the participants are presented in table 5.1. Of the total 200 subjects, 105 (52.5%) were male and 95 (47.5%) were female. Out of 200 respondents, 107 (53.5%) were in the high arsenic exposure group (>400μg/L) and 93 (46.5%) were in the low exposure group (<10μg/L). Respondents residing in houses built with mud were

more likely to consume water containing high concentrations of arsenic. Factors like BMI, participant educational status, and number of rooms in the household did not show marked differences between two groups. Father's smoking was higher in the exposed group.

Table 5.2: Socio-demographic characteristics of study subjects

Characteristics	Total n (%)	Low Exposure Status<10 µg/l n(%)	High Exposure Status>400µg/l n (%)	P-value
Sex				
Boys	105 (52.50)	44 (47.31)	61 (57.01)	0.171
Girls	95 (47.50)	49 (52.69)	46 (42.99)	
Age (years)				
11-13	72 (36.00)	40 (43.01)	32 (29.91)	0.032
14-16	80 (40.00)	38 (40.86)	42 (39.25)	
17-22	48 (24.00)	15 (16.13)	33 (30.84)	
BMI				
<18.5	145 (72.50)	70 (75.27)	75 (70.09)	0.414
>=18.5	55 (27.50)	23 (24.73)	32 (29.91)	
Education (years)				
No	24 (12.00)	8 (8.60)	16 (14.95)	0.533
1-5	46 (23.00)	23 (24.73)	23 (21.50)	
6-10	117 (58.50)	55 (59.14)	62 (57.94)	
11-13	13 (6.50)	7 (7.53)	6 (5.61)	
No. of family members				
2-4	69 (34.50)	33 (35.48)	36 (33.64)	0.772
5-6	95 (47.50)	46 (49.46)	49 (45.79)	
7-8	22(11.00)	9 (9.68)	13 (12.15)	
9+	14 (7.00)	5 (5.38)	9 (8.41)	
Mother's education				
No education	57 (28.50)	23 (24.73)	34 (31.78)	0.007
Primary	87 (43.50)	34 (36.56)	53 (49.53)	
Secondary and above	56 (28.99)	36 (38.71)	20 (18.69)	
Father's education				
No education	61 (30.50)	25 (26.88)	36 (33.64)	<0.001
Primary	68 (34.00)	18 (19.35)	50 (46.73)	
Secondary and above	71 (35.50)	50 (53.76)	21 (19.63)	
Type of house				
Mud	174 (87.00)	73 (78.49)	101 (94.39)	0.004
Mixed	6 (3.00)	5 (5.38)	1 (0.93)	
Concrete	17 (8.50)	13 (13.98)	4 (3.74)	
Tin	3 (1.50)	2 (2.15)	1 (0.93)	
Rooms in the house				
1	12 (6.00)	6 (6.45)	6 (5.61)	0.954
2	64 (32.00)	28 (30.11)	36 (33.64)	
3	96 (48.00)	46 (49.46)	50 (46.730	
4+	28 (14.00)	13 (13.98)	15 (14.02)	
Father smokes				
Yes	116 (58.00)	47 (50.54)	69 (64.49)	0.046
No	84 (42.00)	46 (49.46)	38 (35.51)	

Table 5.2 displays a comparative picture of two renal function parameters between low and high arsenic exposure groups. Participants with high arsenic exposure were more likely to have elevated serum β2MG and the effect was particularly evident in girls. Likewise, the chance of reduced eGFR was found to be more in high arsenic exposure females, but the effect was little in males.

Table 5.3: Comparative figure showing β2MG (age & sex specific) and eGFR status between low and high arsenic exposure groups

Renal function parameters		Total n (%)	Low Exposure Status<10 µg/l n (%)	High Exposure Status>400µg/l n (%)	P-value*
	β2MG status				
	Normal	88 (44.0)	49 (50.7)	39 (36.5)	0.015
	Elevated	112 (56.0)	44 (47.3)	68 (63.6)	
	eGFR status				
	Normal	83 (41.5)	45 (48.4)	38 (35.5)	0.045
	Reduced	117 (58.5)	48 (51.6)	69 (64.5)	
Boys					
	β2MG status				
	Normal	46 (43.8)	21 (47.7)	25 (41.0)	0.313
	Elevated	59 (56.2)	23 (52.3)	36 (59.0)	
	eGFR status				
	Normal	30 (28.6)	14 (38.8)	16 (26.2)	0.341
	Reduced	75 (71.4)	30 (68.2)	45 (73.8)	
Girls					
	β2MG status				
	Normal	42 (44.2)	28 (57.1)	14 (30.4)	0.008
	Elevated	53 (55.8)	21 (42.9)	32 (69.6)	
	eGFR status				
	Normal	53 (55.8)	31 (63.3)	22 (47.8)	0.095
	Reduced	42 (44.2)	18 (36.7)	24 (52.2)	

*One-tailed

Table 5.3 presents multiple logistic regression findings for elevated β2MG. Participants with early life arsenic exposure >400µg/L had nearly 2-fold risk of elevated β2MG compared to low exposure group [OR=1.94; 95% Confidence Interval (CI): 1.10-3.42; p=0.011] in unadjusted model. Adjustment for BMI did not bring about any major change (OR=1.98; 95% CI: 1.12-3.49; p=0.010). The effect was more profound in female participants (OR=3.02, 95% CI: 1.29-7.10; p=0.006) with little evidence in boys (OR=1.36, 95% CI: 0.62-3.00; p=0.222).

Table 5.4: Multiple logistic regression analysis of elevated β2MG (age and sex specific) and early life arsenic exposure status (400+ µg/L compared to less than 10 µg/L)

Elevated β2MG	Un-Adjusted OR (95% CI)	P value**	Adjusted OR (95% CI)*	P value**
All	1.94 (1.10, 3.42)	0.011	1.98 (1.12, 3.49)	0.010
Male	1.31 (0.60, 2.87)	0.246	1.36 (0.62, 3.00)	0.222
Female	3.05 (1.31, 7.10)	0.005	3.02 (1.29, 7.10)	0.006

*Adjusted for BMI; elevated β2MG was defined based on age and sex specific normal reference values.
** One-tailed

Multiple logistic regression (Table 5.4) shows that in the unadjusted model, the children and adolescents with early life arsenic exposure >400µg/L had more than 1.5 times risk of reduced eGFR (<95 mL/min/1.73 $m^{2)}$ compared to the low exposure group [OR=1.70; 95% Confidence Interval (CI): 0.97-3.00; p=0.033]. The risk was nearly 2-fold after adjustment for age, sex and BMI (OR=1.81; 95% CI: 0.97-3.37; p=0.031). The effect was largely confined to female participants (OR=2.89, 95% CI: 1.13-7.38; p=0.013).

Table 5.5: Multiple logistic regression analysis of eGFR<95 mL/min/1.73 m^2 and early life arsenic exposure status (400+ µg/L compared to less than 10 µg/L)

Reduced eGFR (<95 mL/min/1.73 m^2)	Un-adjusted OR (95% CI)	P value**	Adjusted OR (95% CI)*	P value**
All	1.70 (0.97, 3.00)	0.033	1.81 (0.97, 3.37)	0.031
Male	1.31 (0.56, 3.08)	0.266	1.29 (0.53, 3.14)	0.288
Female	1.88 (0.83, 4.26)	0.066	2.89 (1.13, 7.38)	0.013

* Adjusted for age, sex, BMI
** One-tailed

Discussion

To the best of our knowledge, this is the first study evaluating renal function in older children over age 11 and adolescents with high water arsenic exposure in their early life. We found modest reduction in renal function. Children and adolescents with an early life arsenic exposure level of >400 µg/L had nearly 2-fold higher risk of elevated β2MG and reduced eGFR after adjustment for potential confounders. The effect was largely confined to female participants.

Chronic exposure to arsenic via drinking water has been linked to chronic kidney diseases in adults (6, 26, 27). A study in Taiwan reported an association between urinary arsenic concentrations and decrements in renal function assessed by two parameters, β2MG and eGFR. After adjustment for potential confounders, the odds for abnormal β2MG (> 0.154 mg/L) and reduced eGFR < 90 mL/ min/ (1.73 m2) was increased around 2 times in subjects with urinary arsenic >75 µg/g creatinine compared to ≤35µg/g creatinine, and the relationship was dose-dependent (28). Elevated risk of proteinuria, a marker of renal function has been observed in an adult population in Bangladesh exposed to wide range of arsenic from drinking water. Adjusted prevalence odds ratios (PORs) for proteinuria were 1.00 (reference group), 1.01 [95% confidence interval (CI): 0.79–1.31], 1.33 (95% CI: 1.04–1.70), 1.54 (95% CI: 1.22-1.96), 1.65 (95% CI: 1.31–2.09) (P for trend <0.01) for the well water arsenic levels ≤7, 8–39, 40–91, 92–179 and 180–864 µg/l respectively (13).

A recent cross-sectional study on adults in an arsenic endemic area in Bangladesh reported marginal inverse association between total urinary arsenic (uAs) and serum cystatin C derived

eGFR. The mean difference in eGFR was -2.55 ml/min/1.73 m^2 (b=-2.55, p=0.08) per unit increase in total uAs (29).

Increased mortality from chronic kidney diseases including renal failure has been observed in cohorts exposed to high concentration of (>800 µg/L) in utero or early postnatal life in Antofagasta, Chile. Death from chronic renal diseases was increased around 2-fold [SMR=2.0; 95% Confidence Interval (CI): 1.5 to 2.8] in young adults aged 30-59 born before or during the high-exposure period of 1958–1970 (16)

A prospective study among children in Bangladesh reported a marginally inverse relationship between early life arsenic exposure and renal function in childhood in the age rang 4.4-5.4 years. For one unit increase in urinary arsenic at 18 months of age the mean difference for plasma Cystatin C derived estimated glomerular filtration rate (eGFR) was -33.4 ml/min/1.73m^2 (95% CI: -70.2, 3.34; P: 0.08) (18).

However, the mechanism of arsenic induced renal toxicity is not well understood. The kidney may be a target organ as arsenic is primarily excreted through the kidney, and proximal tubules may be more sensitive because of their high reabsorptive capacity (30). Prenatal arsenic exposure in mice has found to induce renal hyperplasia in the offspring during adulthood (31). Mice exposed to arsenic have shown oxidative stress mediated DNA damage in kidney tissue (32). Arsenic increases reactive oxygen species (ROS) and induces oxidative stress that may lead to renal damage by increasing the expression of hemeoxygenase-1 and Mitogen-Activated Protein Kinases (MAPK) (33). Arsenic induced inflammation (34, 35) oxidative stress (36) and apoptosis (37) might play a role in renal toxicity.

Conclusion

Our study demonstrated some reduction in renal function in female children and adolescents exposed to high concentration of arsenic in early life but very little effect in males. Further studies are needed to confirm renal effects following early life exposure to arsenic in water, and to explore the puzzling sex differences we found.

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Chapter 6

Summary and Conclusion

This prospective cohort study provides evidence that early life exposure to high concentration of inorganic arsenic in drinking water is related to respiratory, cardiovascular and renal effects in children and adolescents. The key findings of my doctoral thesis are presented in the table below.

Table 6.1: Table showing the key findings from the doctoral thesis

	Overall	Boys	Girls
Major respiratory findings		**POR (95% CI); P value**	
*Number of wheezing attacks (1-3 times)/week	1.51 (0.57-4.00); 0.21	4.99 (1.00-24.90); 0.03	0.31 (0.06-1.79); 0.91
*Wheezing after exercise	2.13 (0.79-5.73); 0.07	4.14 (1.05-16.40); 0.02	0.64 (0.12-3.40); 0.70
*Woken up with tightness of chest	1.58 (0.60-4.13); 0.18	5.01 (1.00-25.00); 0.02	0.42 (0.09-1.98); 0.86
		Co-eff (95% CI); P value	
**FEV1	-24.4 (-104.50, 55.7); 0.28	-117.3 (-246.5, 11.8); 0.04	109.2 (27.4, 191.0); 0.99
**FVC	-39.2 (-124.10, 45.6); 0.18	-135.2 (269.9, -0.37); 0.02	91.1 (-2.59, 184.7); 0.97
Blood Pressure findings		**POR (95% CI); P value**	
***Prehypertension	1.68 (0.65-4.35); 0.14	0.85 (0.23-3.15); 0.60	3.93 (0.79-19.6); 0.05
Renal function		**OR (95% CI); P value**	
*****Elevated β2MG	1.98 (1.12, 3.49); 0.010	1.36 (0.62, 3.00); 0.222	3.02 (1.29, 7.10); 0.006
*****Reduced eGFR	1.81 (0.97-3.37); 0.03	1.29 (0.53-3.14); 0.29	2.89 (1.13-7.38); 0.01

*Prevalence Odds Ratio (POR) for respiratory symptoms in high arsenic exposure group (>400µg/l) compared to low (<10µg/l) adjusted for age, sex, father's smoking status and rooms in the house in a multiple logistic regression model.

**Regression co-efficients of lung function parameters in high exposure participants adjusted for age, sex, height, weight, father's smoking status and rooms in the house obtained by multiple linear regression.

***Prevalence Odds Ratio (POR) for prehypertension in the high exposure group compared to the low exposure group adjusted for age, sex, BMI, father's smoking status and rooms in the house obtained by multiple logistic regression.

**** Odds Ratio (OR) for elevated β2MG (age and sex specific) in the high exposure group adjusted for BMI obtained by multiple logistic regression.

*****Odds Ratio (OR) for reduced eGFR in the high exposure group adjusted for age, sex, BMI obtained by multiple logistic regression.

This is the first study that explored the respiratory effects in adolescents with early life exposure. We found that early life arsenic exposure via drinking water increased the risk of respiratory symptoms including impairment of lung function in males (Chapter 3). Finding respiratory effects was expected based on our earlier findings for this cohort obtained about four years ago (see the paper in the Appendix-7).

In our original cohort study, we had found marked increase in respiratory symptoms in children exposed to inorganic arsenic in their early life but there was little effect on lung function. Increased risk of respiratory symptoms was evident both in boys and girls (1). However, the present study found elevated risks of respiratory symptoms only among males while there was no effect of early life arsenic exposure among females. Interestingly, we observed reduced lung function among the male participants which was absent in the original cohort study.

However, we were surprised that there is now no evidence of respiratory effects in girls. This suggests that with reductions in arsenic exposure, girls may recover from respiratory effects more rapidly than boys. Although these sex specific findings are somewhat surprising, a number of studies have reported gender differences in respiratory effects of arsenic (2-6).

However such findings need to be confirmed in other populations, and further follow up of this cohort will show if respiratory effects persist in boys, and if girls have permanently recovered.

In the first cohort study, we investigated only the respiratory effects in children exposed to arsenic in early life. However, as there is growing evidence that early life exposure to arsenic increases the risk of cardiovascular and kidney diseases, the present study intended to identify

the effects of arsenic exposure through drinking water on blood pressure and renal function in adolescents.

We found early life arsenic exposure to be associated with prehypertension in girls. After adjusting for potential confounders, there was an elevated risk for pre-hypertension among girls exposed to arsenic >400 µg/L in early life (POR=3.93, 95% CI: 0.79-19.6, p=0.05). The effect was evident only in females as presented in Chapter 4. Contrary to the respiratory effects, this finding was confined to girls. Girls with early life exposure had a four-fold higher prevalence of prehypertension compared to girls without early life exposure, but there was no effect in exposed boys. A couple of studies also reported higher cardiovascular risk among women compared to men (7, 8). However, this sex specific effect needs to be validated by further studies.

We found modest reduction in kidney function measured by serum β2MG and eGFR in girls exposed to elevated arsenic concentrations in early life but little effect in boys as presented in chapter 5. Risk of reduced renal function increased 3-fold in females (OR=3.02, 95% CI: 1.29-7.10; p=0.006). This finding also needs further evaluation.

If elevated blood pressure can be diagnosed at prehypertension stage, measures could be taken to prevent further progression to hypertension and cardiovascular morbidity and mortality. Similarly, if renal function impairment can be detected at the early stage before the substantial reduction in kidney function, the morbidity and death from chronic kidney diseases might be prevented.

We plan to follow the full cohort of 600 children to identify early stage respiratory, cardiovascular, metabolic and renal effects with biomarkers.

To conclude, this study suggests that early life arsenic is associated with long term health consequences in children and adolescents in rural Bangladesh. Although arsenic mitigation activities have been in action, a recent nation-wide survey in Bangladesh shows that 20 million people are still drinking water with an arsenic concentration above the national standard of 50µg/L. If the WHO limit of 10µg/L is considered, the figure would be 45 million, over one-fourth of the country's total population (9). A recent study reported that 27% of children in Matlab are still taking tube well water with arsenic concentration above the national standard despite intensive mitigation efforts in this area (10).

Considering the magnitude of arsenic exposure status and the long term health effects, it is imperative to introduce effective, sustainable and affordable solutions immediately. Even if the exposure is stopped now and people switch to safe water, this generation might have to face latent effects of early life exposure, but the next generation would be free from arsenic induced health hazards. However, if we fail to control the exposure right now, we would allow future generations to be at risk of population-wide chronic morbidity and mortality (9). Although drinking water from deep tube-wells has been considered an immediate solution, evidence suggests that mere installation of deep tube-wells is not sufficient to reduce wide spread exposure for long periods (11). National and international stakeholders should come forward with a robust and pragmatic plan to mitigate arsenic contamination in Bangladesh on an urgent basis. We feel it would be useful to make the people aware of the deleterious effects of arsenic exposure through drinking water and motivate them to switch to safe water immediately.

Arsenic in drinking water is still a prevailing public health concern in rural Bangladesh and demands national and international attention.

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Appendix

Appendix-1a: Consent form (English)

International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b)

Written Assent Form (For children 11-17 years)

Protocol Number: PR-12012

Protocol Title: E*xposure to arsenic in utero and early life and effects on respiratory diseases, hypertension, diabetes and renal function in children and adolescents in rural Matlab, Bangladesh"*

Principal Investigator: Md Yunus

Purpose of the research

Thank you very much for being in our earlier study. We now have evidence to believe that arsenic in drinking water may cause various chronic illnesses including chronic lung diseases, hypertension, diabetes and chronic renal disease. Many studies have looked at the effects of arsenic in drinking water in adults, but there have been few studies on the impacts of early life exposure on risks of diseases such as hypertension, chronic renal failure, or pulmonary effects like reduced lung function or respiratory symptoms in children and adolescents. The purpose of this study is to look at how early life exposure of arsenic through drinking water affects the lungs, kidney, blood pressure and blood sugar for participants aged 10 to 20.

Why selected

In our earlier study we looked at 600 children for skin lesions (changes in the skin), chronic respiratory symptoms including lung function and measured the level of arsenic in their drinking water. Using the information from that study, we have chosen two groups of children for this study. Among 300 exposed children, we will select 100 children with highest exposure during their mother's pregnancy and in the child's early life. Another 100 gender and age-matched children will be randomly selected from the 300 unexposed children. All children chosen for the study are between 10 and 20 years old. You are within this age range.

What is expected from the participants?

If you agree to be in our study, our Field Research Assistant (FRA) will ask you a list of questions on your drinking water sources, dietary history, socioeconomic information etc. This will take place at your home. This might take an hour. You will also be invited to visit Matlab hospital for about one and a half hours. You would need to come to the hospital without taking any food in the morning g At the hospital, a trained doctor will take your medical history; carry out a physical examination including measuring blood pressure and test how well your lung works. To test your lung function, you will be asked to blow into a tube as hard as you can, like blowing up a balloon as before. Your skin will also be examined for changes caused by arsenic. If it seems like there changes to your skin, photographs will be taken of your skin so that other doctors can see them. Under aseptic precautions, a physician/nurse will take 5 ml (1 teaspoon)

of venous blood for measuring HbA1c to detect diabetes, serum CC16 (a marker of damage of lung cells) and β-2 microglobulin (a marker of kidney function). A trained physician/nurse will also collect secretions from inside of your mouth (inside both cheeks) using a small brush for collection of cells inside the mouth. We will ask you to give us a 60 ml urine sample for measurement of arsenic concentration and collection of urothelial cells (mainly cells from the bladder which are normally present in urine). A spot urinalysis will be done by strip to detect any presence of protein and sugar Both cells from the mouth (buccal cells) and bladder will be tested to look for changes due to arsenic. Water samples from the tube wells you used since our last visit will also be collected to test for arsenic concentrations.

Risk and benefits

The risk from being in this study is very small. A trained doctor will do the physical examination and the lung function test. The physical exam and lung test will not hurt you. The general medical examination by a doctor may be beneficial to you. Blood samples will be taken aseptically; you will just feel slight pain.

There are no benefits to you for being in this study. All costs for travel to the Matlab centre and the wage loss (Tk-300.00) for that day will be paid for by the study. During the waiting time at the centre, drinks and snacks will be provided. You will be given "educational materials in a bag" as a gift for being in the study.

Privacy, anonymity and confidentiality

We would like you to know that none of the information collected from you and your family , including information from the physical examination, will be passed on to anybody else. Only the researchers of this study and Ethical Review Committee will be able to look at the information. The study records will not have your name on them. Each person will have a code number and this number will be used instead of the name. Your name or any information that could be used to identify you (find out who you are) will not be published or shared with anyone else.

Future use of information

The information collected in this study might be used in the future to continue studying the health effects of arsenic in water, but again information that could be used to identify you will not be shared.

Right not to participate and withdraw

Your participation is voluntary. This means that you may choose not to be in the study. You may also choose to stop being in the study at any time, even after you have said that you want to be in the study. No matter what you choose, you and other family members will receive the same quality care at icddr,b facilities. You do not have to answer any question that makes you feel uncomfortable.

You may ask any question about this study and we shall be happy to answer it. For further questions you may call Dr. Md Yunus, Principal Investigator of this study (mobile no.01713093872) or Dr. Md Al Fazal Khan, Matlab hospital (mobile no.017130024702). If you feel that you have been treated unfairly or have been hurt by joining in this study you may also call M. A. Salam Khan, Assistant coordination Manager, IRB at icddr,b. The phone contact number of Mr. Khan Rahman is 02- 9886498 (Office) or 02-8860523-32 Ext 3206; Mobile no.01711428989.

Do you have any questions? Yes No

Do you agree to be in the study? Yes No

Now we are inviting you to be in our study. If you agree to be in the study, please show us that you agree by putting your signature (your name) or your left thumbprint in the space provided below.

Thank you for your cooperation

_____	_____
Signature or left thumbprint of participant	Date
_____	_____
Signature or left thumbprint of the parent/guardian	Date
_____	_____
Signature of the PI or his/her representative	Date

(Note): In case of representative of the PI, she/he shall put her/his full name and designation and then sign

International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b)

Written Consent Form (18-20years)

Protocol Number: PR-12012
Protocol Title: E*xposure to arsenic in utero and early life and effects on respiratory diseases, hypertension, diabetes and renal function in children and adolescent in rural Matlab, Bangladesh"*
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What is expected from the participants?

If you agree to be in our study, our Field Research Assistant (FRA) will ask you a list of questions on your drinking water sources, dietary history, socioeconomic information etc. This will take place at your home. This might take an hour. You will also be invited to visit Matlab hospital for about one and a half hours. You would need to come to the hospital without taking any food in the morning g At the hospital, a trained doctor will take your medical history; carry out a physical examination including measuring blood pressure and test how well your lung works. To test your lung function, you will be asked to blow into a tube as hard as you can, like blowing up a balloon as before. Your skin will also be examined for changes caused by arsenic. If it seems like there changes to your skin, photographs will be taken of your skin so that other doctors can see them. Under aseptic precautions, a physician/nurse will take 5 ml (1 teaspoon) of venous blood for measuring HbA1c to detect diabetes, serum CC16 (a marker of damage of lung cells) and β-2 microglobulin (a marker of kidney function). A trained physician/nurse will

also collect secretions from inside of your mouth (inside both cheeks) using a small brush for collection of cells inside the mouth. We will ask you to give us a 60 ml urine sample for measurement of arsenic concentration and collection of urothelial cells (mainly cells from the bladder which are normally present in urine). A spot urinalysis will be done by strip to detect any presence of protein and sugar. Both cells from the mouth (buccal cells) and bladder will be tested to look for changes due to arsenic. Water samples from the tube wells you used since our last visit will also be collected to test for arsenic concentrations.

Risk and benefits

The risk from being in this study is very small. A trained doctor will do the physical examination and the lung function test. The physical exam and lung test will not hurt you. The general medical examination by a doctor may be beneficial to you. Blood samples will be taken aseptically; you will just feel slight pain.

There are no benefits to you for being in this study. All costs for travel to the Matlab centre and the wage loss (Tk-300.00) for that day will be paid for by the study. During the waiting time at the centre, drinks and snacks will be provided. You will be given "educational materials in a bag" as a gift for being in the study.

Privacy, anonymity and confidentiality

We would like you to know that none of the information collected from you and your family , including information from the physical examination, will be passed on to anybody else. Only the researchers of this study and Ethical Review Committee will be able to look at the information. The study records will not have your name on them. Each person will have a code number and this number will be used instead of the name. Your name or any information that could be used to identify you (find out who you are) will not be published or shared with anyone else.

Future use of information

The information collected in this study might be used in the future to continue studying the health effects of arsenic in water, but again information that could be used to identify you will not be shared.

Right not to participate and withdraw

Your participation is voluntary. This means that you may choose not to be in the study. You may also choose to stop being in the study at any time, even after you have said that you want to be in the study. No matter what you choose, you and other family members will receive the same quality care at icddr,b facilities. You do not have to answer any question that makes you feel uncomfortable.

You may ask any question about this study and we shall be happy to answer it. For further questions you may call Dr. Md Yunus, Principal Investigator of this study (mobile no.01713093872) or Dr. Md Al Fazal Khan, Matlab hospital (mobile no.017130024702). If you feel that you have been treated unfairly or have been hurt by joining in this study you may also call M. A. Salam Khan, Assistant coordination Manager, IRB at icddr,b. The phone contact number of Mr. Khan Rahman is 02- 9886498 (Office) or 02-8860523-32 Ext 3206; Mobile no.01711428989.

Do you have any questions? Yes No

Do you agree to be in the study? Yes No

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Thank you for your cooperation

_____	_____
Signature or left thumbprint of participant	Date
_____	_____
Signature or left thumbprint of the witness	Date
_____	_____
Signature of the PI or his/her representative	Date

(Note): In case of representative of the PI, she/he shall put her/his full name and designation and then sign

Appendix- 1b: Consent form (English) (Pdf)

icddr,b
KNOWLEDGE FOR
GLOBAL LIFESAVING SOLUTIONS

International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b)
Written Assent Form (For children 11-17 years)

Protocol Number: PR-12012
Protocol Title: *Exposure to arsenic in utero and early life and effects on respiratory diseases, hypertension, diabetes and renal function in children and adolescents in rural Matlab, Bangladesh"*
Principal Investigator: Md Yunus

Purpose of the research

Thank you very much for being in our earlier study. We now have evidence to believe that arsenic in drinking water may cause various chronic illnesses including chronic lung diseases, hypertension, diabetes and chronic renal disease. Many studies have looked at the effects of arsenic in drinking water in adults, but there have been few studies on the impacts of early life exposure on risks of diseases such as hypertension, chronic renal failure, or pulmonary effects like reduced lung function or respiratory symptoms in children and adolescents. The purpose of this study is to look at how early life exposure of arsenic through drinking water affects the lungs, kidney, blood pressure and blood sugar for participants aged 10 to 20.

Why selected

In our earlier study we looked at 600 children for skin lesions (changes in the skin), chronic respiratory symptoms including lung function and measured the level of arsenic in their drinking water. Using the information from that study, we have chosen two groups of children for this study. Among 300 exposed children, we will select 100 children with highest exposure during their mother's pregnancy and in the child's early life. Another 100 gender and age-matched children will be randomly selected from the 300 unexposed children. All children chosen for the study are between 10 and 20 years old. You are within this age range.

What is expected from the participants?

If you agree to be in our study, our Field Research Assistant (FRA) will ask you a list of questions on your drinking water sources, dietary history, socioeconomic information etc. This will take place at your home. This might take an hour. You will also be invited to visit Matlab hospital for about one and a half hours. You would need to come to the hospital without taking any food in the morning g At the hospital, a trained doctor will take your medical history; carry out a physical examination including measuring blood pressure and test how well your lung works. To test your lung function, you will be asked to blow into a tube as hard as you can, like blowing up a balloon as before. Your skin will also be examined for changes caused by arsenic. If it seems like there changes to your skin, photographs will be taken of your skin so that other doctors can see them. Under aseptic precautions, a physician/nurse will take 5 ml (1 teaspoon) of venous blood for measuring HbA1c to detect diabetes, serum CC16 (a marker of damage of lung cells) and β-2 microglobulin (a marker of kidney function). A trained physician/nurse will also collect secretions from inside of your mouth (inside both cheeks) using a small brush for collection of cells inside the mouth. We will ask you to give us a 60 ml urine sample for measurement of arsenic concentration and collection of urothelial cells (mainly cells from the bladder which are normally present in urine). A spot urinalysis will be done by strip to detect any presence of protein and sugar Both cells from the mouth (buccal cells) and bladder will be tested to look for changes due to arsenic. Water samples from the tube wells you used since our last visit will also be collected to test for arsenic concentrations.

icddr,b
KNOWLEDGE FOR
GLOBAL LIFESAVING SOLUTIONS

International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b)
Written Consent Form (18-20years)

Protocol Number: PR-12012
Protocol Title: *Exposure to arsenic in utero and early life and effects on respiratory diseases, hypertension, diabetes and renal function in children and adolescent in rural Matlab, Bangladesh"*
Principal Investigator: Md Yunus

Purpose of the research

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Appendix-2a: Consent form (Bangla)

আন্তর্জাতিক উদারাময় গবেষণা কেন্দ্র, বাংলাদেশ
লিখিত সম্মতি পত্র (Assent)(১১-১৭ বৎসরের ছেলে/মেয়েদের জন্য)

গবেষণা নম্বর: PR- 12012

গবেষণার শিরোনাম: **: গর্ভাবস্থায় এবং খুব অল্প বয়সে আর্সেনিকের দোষন এবং বাচ্চাদের ও কিশোর- কিশোরীদের স্বাস-প্রস্বাসজনিত অসুখ, রক্তচাপ, ডাইবেটিস এবং মুত্রাশয়ের কার্যকারিতার উপর প্রভাব।**

প্রধান গবেষক: ডাঃ মোহাম্মদ ইউনুস

গবেষণার উদ্দেশ্য:

আমাদের পূর্বের আর্সেনিক গবেষণায় অংশগ্রহণ-এর জন্য আপনাকে ধন্যবাদ। এখন আমাদের কাছে বিস্বাসযোগ্য কিছু তথ্য-প্রমান আছে যে, আর্সেনিকযুক্ত পানি পান করলে ফুসফুসের রোগ, উচ্চ রক্তচাপ ও ডাইবেটিস সহ বিভিন্ন ধরনের দীর্ঘমেয়াদী অসুখ হতে পারে। আর্সেনিকযুক্ত পানি পান করলে বয়স্কদের উপর কি প্রতিক্রিয়া হতে পারে এ নিয়ে অনেক গবেষনা করা হয়েছে কিন্তু অল্প বয়স থেকে আর্সেনিকযুক্ত পানি পান করার ফলে বাচ্চাদের ও কিশোর-কিশোরীদের বিভিন্ন রোগের ঝুকি যেমন উচ্চরক্তচাপ, মুত্রগ্রন্থির দীর্ঘমেয়াদী অকার্য্যকারীতা, ফুসফুসের উপর প্রভাব যেমন ফুসফুসের কার্য্যকারিতা কমে যাওয়া অথবা স্বাসপ্রস্বাসজনিত লক্ষনের ক্ষেত্রে অল্প সংখ্যক গবেষণা করা হয়েছে। এই গবেষণার উদ্দেশ্য হল খুব অল্প বয়সে খাবার পানিতে আর্সেনিক দূষণের ফলে ১০ - ২০ বৎসর বয়সের মানুষের ফুসফুস, মুত্রগ্রন্থি, রক্তচাপ ও রক্তের চিনির উপর কিভাবে প্রভাব ফেলে তা দেখা।

নির্বাচনের কারণ:

পূর্বের আর্সেনিক গবেষণায় আমরা ৬০০ বাচ্চার উপর আর্সেনিকজনিত চামড়ার ক্ষত, ফুসফুসের কার্যকারিতা সহ দীর্ঘমেয়াদী স্বাস-প্রস্বাসজনিত রোগের লক্ষণ এবং তাদের খাবার পানিতে আর্সেনিকের মাত্রা নির্ধরণ করেছি। উক্ত গবেষণার তথ্য ব্যবহার করে আমরা দুই দল বাচ্চা বেছে নিয়েছি। আর্সেনিকের মাত্রা বেশী ছিল এমন ৩০০ জন বাচ্চা থেকে আমরা এমন ১০০ জন বাচ্চা নির্ধারণ করব যাদের মাযের পেটে থাকা কালীন অবস্থায় এবং অল্প বয়সের সময় সবচেয়ে উচু মাত্রায় আর্সেনিক ছিল। লিঙ্গ ও বয়স অনুরূপ হওয়া আরো ১০০ বাচ্চা যাদের আর্সেনিকের মাত্রা প্রতি লিটারে ১০ মাইক্রোগ্রাম এর কম ছিল তাদের থেকে নৈর্ব্যক্তিক উপায়ে (লটারী করে) নির্ধারণ করব। সমস্ত অংশগ্রহণকারীদের বয়স ১০ - ২০ বৎসরের মধ্যে হতে হবে। আপনি যেকোনো এক দলে এই বয়স সীমার মাঝে আছেন।

অংশগ্রহণকারীর নিকট থেকে প্রত্যাশা:

আপনি যদি আমাদের এ গবেষণায় অংশগ্রহণ করতে রাজি হন তবে আমাদের মাঠ গবেষণা কর্মী আপনাকে আপনার খাবার পানির উৎস সমুহ, খাদ্য গ্রহণের ইতিহাস, আর্থসামাজিক অবস্থা ইত্যাদি বিষয়ের উপর কিছু প্রশ্ন জিজ্ঞাসা করবেন। এটা করতে এক ঘন্টার মত সময় লাগতে পারে। আপনাকে প্রায় এক ঘন্টা ৩০ মিনিটের জন্য মতলব হাসপাতালে আসতে হবে। হাসপাতালে একজন প্রশিক্ষণপ্রাপ্ত ডাক্তার আপনার স্বাস্থ্য সম্পর্কে ইতিহাস সংগ্রহ এবং রক্তচাপ মাপা ও ফুসফুসের কার্যকারিতা সহ শারীরিক পরীক্ষা করবেন। ফুসফুসের কার্য্যকারিতা পরীক্ষা করার সময় পূর্বের মত আপনাকে একটি নলের মধ্যে খুব জোরে ফুঁ দিতে হবে যেমন করে বেলুন ফুলানো হয়। আর্সেনিকের জন্য চামড়ায় কোনো পরিবর্তন হয়েছে কিনা তাও পরীক্ষা করা হবে। যদি চামড়ায় সন্দেহজনক কোনো পরিবর্তন দেখা যায় তাহলে ঐস্থানের চামড়ার ছবি তোলা হবে যাতে অন্যান্য ডাক্তারগণ দেখতে পারে। জীবানু না ছড়ানোর ব্যাপারে সমস্ত সতর্কতামূলক ব্যবস্থা গ্রহণ পূর্বক একজন ডাক্তার বা নার্স আপনার রক্তের অভুক্ত অবস্থায় চিনির পরিমান, রক্তে সিসি ১৬ (ফুসফুসের কোষের বিষাক্ততা বুঝার নির্দেশক) পরিমাপ করার জন্য আপনার শরীরের শিরা থেকে ৫ মিঃ লিঃ (১ চামচ) পরিমান রক্ত সংগ্রহ করবেন। একজন প্রশিক্ষণ প্রাপ্ত ডাক্তার বা নার্স মুখের কোষ (Buccal cells) সংগ্রহ করার জন্য একটি মুখের সোয়াব (cyto brush) দ্বারা আপনার দুই গালের ভিতরের দিক থেকে মুখের লালা সংগ্রহ করবেন। আর্সেনিকের পরিমাণ মাপার জন্য এবং মুত্রথলীর কোষ (urothelial cell) সংগ্রহের জন্য আমরা আপনাকে ৬০ মিঃ লিঃ প্রস্রাব দেবার জন্য অনুরোধ করব। মুখের কোষ (Buccal cells) এবং মুত্রথলীরকোষ (urothelial cell) উভয়ই DNA বা বংশানুগতি নির্ধারনের ক্ষেত্রে এই পর্য়ন্ত কোনো প্রকার পরিবর্তন হয়েছে তা পরীক্ষা করার জন্য।

গবেষনার ঝুকি এবং অংশগ্রহনের সুবিধাঃ

এই গবেষনায় অংশগ্রহনে ঝুকি খুবই সামান্য। একজন অভিজ্ঞ এবং প্রশিক্ষণপ্রাপ্ত চিকিৎসক আপনার শারীরিক পরীক্ষা এবং ফুসফুসের কার্য্যকারিতা পরীক্ষা করবেন। শারীরিক এবং ফুসফুসের কার্য্যকারিতা পরীক্ষায় আপনার কোনো আঘাত লাগবেনা। একজন ডাক্তার দ্বারা আপনার সাধারন স্বাস্থ্য পরীক্ষা আপনার জন্য মঙ্গলজনক হতে পারে। জীবানু না ছড়ানোর ব্যাপারে সতর্কতামূলক ব্যবস্থা গ্রহণ করিয়া রক্ত সংগ্রহ করা হবে এবং এতে আপনি সামান্য ব্যাথা অনুভব করতে পারেন।

এই গবেষনায় অংশ্রগ্রহনের জন্য কোন সরাসরি সুবিধাদি নেই। মতলব সেন্টারে যাতায়াতের সমস্ত খরচ এবং ঐ দিনের মজুরী লোকসান বাবদ ৩০০ টাকা এই গবেষণার পক্ষ থেকে আপনাকে দেয়া হবে। মতলব সেন্টারে অপেক্ষাকালীন সময়ে আপনাকে নাস্ড়া এবং পানীয় দেওয়া হবে। এই গবেষণায় অংশগ্রহণের জন্য আপনাকে একটি ব্যাগে প্রয়োজনীয় শিক্ষামূলক জিনিষপত্র উপহার হিসাবে দেওয়া হবে।

গোপনীয়তা, নামহীনতা এবং বিশ্বস্ড়তা :

আমারা আপনাকে জানাতে চাই যে, আপনার স্বাস্থ্যগত পরীক্ষার ফলাফল সহ আপনার এবং আপনার পরিবার থেকে সংগ্রহ করা কোন তথ্য অন্য কাউকে দেওয়া হবে না। শুধূ মাত্র এ গবেষনার গবেষকগণ এবং নীতি পর্যালোচনা কমিটি (ই আর সি) এসব তথ্য দেখতে পারবেন। এই গবেষণায় কোন কাগজ-পত্রে আপনার নাম থাকবে না । প্রত্যেক ব্যক্তির জন্য একটি সাংকেতিক নম্বর দেয়া হবে এবং নামের বদলে এই সাংকেতিক নম্বর ব্যবহার করা হবে। আপনার নাম অথবা কোনো তথ্য যা দিয়ে আপনাকে সনাক্ত করা যায় তাহা প্রকাশ করা হবে না অথবা অন্য কাউকে দেওয়া হবে না।

ভবিষ্যতে ব্যবহারযোগ্য তথ্যঃ

এ গবেষণায় সংগ্রহ করা তথ্য পানিতে আর্সেনিক দূষনের দ্বারা স্বাস্থ্যের উপর প্রভাব সম্পর্কিত গবেষনার কাজে ভবিষ্যতে ব্যবহার করা যেতে পারে। তবে তখনো আপনাকে সনাক্ত করা যায় এমন কোন তথ্য অন্য কাউকে দেওয়া হবে না।

অংশগ্রহণ না করার এবং প্রত্যাহার করার অধিকারঃ

এই গবেষনায় অংশগ্রহন আপনার স্বেচ্ছাধীন। এটার অর্থ এই যে আপনি এই গবেষণায় অংশগ্রহণ নাও করতে পারেন। আপনি যেকোনো সময় এই গবেষনায় অংশ গ্রহণ থেকে বিরত থাকতে পারেন এমনকি যদিও আপনি বলে ছিলেন যে আপনি এটাতে অংশগ্রহন করতে চান।আপনি যাই সিদ্ধান্ত নেন না কেন আপনি এবং আপনার পরিবারের অন্য সদস্যগন আই সি ডি ডি আর বি চিকিৎসা কেন্দ্রগুলো থেকে একই মানের সেবা পাবেন। আপনি কোন অস্বস্ড়ি বোধ করেন এমন কোনো প্রশ্নের উত্তর আপনাকে দিতে হবে না। আপনি এই গবেষনার ব্যাপারে যে কোন প্রশ্ন করতে পারেন এবং আমরা **খুশি মনে** এর উত্তর দেব। এই গবেষণা প্রসঙ্গে আপনি যদি আরও কিছু জানতে চান তাহলে ডাঃ মোঃ ইউনুস, এই গবেষণা কাজের প্রধান গবেষক (মোবাইল নম্বর ০১৭১৩০৯৩৮৭২) অথবা ডাঃ মোঃ আল্ ফজল খান (মোবাইল নম্বর ০১৭১৩০০২৪৭০২) মতলব হাসপাতাল এর সঙ্গে যোগাযোগ করতে পারেন। আপনি যদি মনে করেন যে আপনার সঙ্গে কোন অন্যায় আচরণ করা হয়েছে অথবা এই গবেষণায় যোগদানের ফলে আপনি আঘাতপ্রাপ্ত হয়েছেন তাহলে আপনি জনাব এস এ সালাম খান, সহকারী সমন্বয়কারী ব্যবস্থাপক, আই সি ডি ডি আর বি প্রাতিষ্ঠানিক পর্যালোচনা বোর্ড এর সঙ্গে যোগাযোগ করতে পারেন। জনাব সালাম খানের সঙ্গে যোগাযোগের নাম্বার হল ০২-৯৮৮৪৯৪ (অফিস) অথবা ০২-৮৮৬০৫২৩-৩২ এক্সটেনশন ৩২০৬ অথবা মোবাইল নং - ০১৭১১৪২৮৯৮৯।

আপনার কি কোন প্রশ্ন আছে?	হ্যাঁ	না
আপনি কি এ গবেষণায় অংশগ্রহণের জন্য সম্মত আছেন?	হ্যাঁ	না

এখন আমরা আপনাকে এ গবেষণায় অংশগ্রহনের জন্য আমন্ত্রণ জানাচ্ছি। আপনি যদি এ গবেষণায় অংশগ্রহণ করতে রাজী থাকেন তাহলে অনুগ্রহপূর্বক নীচে নির্ধারিত স্থানে আপনার স্বাক্ষর অথবা বাম হাতের বৃদ্ধাঙ্গুলির টিপ দিন।

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অংশগ্রহনকারীর স্বাক্ষর অথবা বাম হাতের বৃদ্ধাঙ্গুলির টিপ তারিখ

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অভিভাবকের স্বাক্ষর অথবা বাম হাতের বৃদ্ধাঙ্গুলির টিপ তারিখ

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প্রধান গবেষক/প্রতিনিধির স্বাক্ষর তারিখ

(নোট: প্রধান গবেষক এর প্রতিনিধির ক্ষেত্রে পূর্ণাঙ্গ নাম, পদবী এবং স্বাক্ষর উলেণ্ঢখ করতে হবে।)

আন্তর্জাতিক উদারাময় গবেষণা কেন্দ্র, বাংলাদেশ
লিখিত সম্মতি পত্র (১৮-২০ বৎসরের জন্য)

গবেষণা নম্বর: PR- 12012

গবেষণার শিরোনাম: **: গর্ভাবস্থায় এবং খুব অল্প বয়সে আর্সেনিকের দোষন এবং বাচ্চাদের ও কিশোর- কিশোরীদের স্বাস-প্রস্বাসজনিত অসুখ, রক্তচাপ, ডাইবেটিস এবং মুত্রাশয়ের কার্যকারিতার উপর প্রভাব।**

প্রধান গবেষক: ডাঃ মোহাম্মদ ইউনুস

গবেষণার উদ্দেশ্য:

আমাদের পূর্বের আর্সেনিক গবেষণায় অংশগ্রহণ-এর জন্য আপনাকে ধন্যবাদ। এখন আমাদের কাছে বিস্বাসযোগ্য কিছু তথ্য-প্রমান আছে যে, আর্সেনিকযুক্ত পানি পান করলে ফুসফুসের রোগ, উচ্চ রক্তচাপ ও ডাইবেটিস সহ বিভিন্ন ধরনের দীর্ঘমেয়াদী অসুখ হতে পারে। আর্সেনিকযুক্ত পানি পান করলে বয়স্কদের উপর কি প্রতিক্রিয়া হতে পারে এ নিয়ে অনেক গবেষনা করা হয়েছে কিন্তু অল্প বয়স থেকে আর্সেনিকযুক্ত পানি পান করার ফলে বাচ্চাদের ও কিশোর-কিশোরীদের বিভিন্ন রোগের ঝুকি যেমন উচ্চরক্তচাপ, মুত্রগ্রন্থির দীর্ঘমেয়াদী অকার্য্যকারীতা, ফুসফুসের উপর প্রভাব যেমন ফুসফুসের কার্য্যকারিতা কমে যাওয়া অথবা স্বাসপ্রস্বাসজনিত লক্ষনের ক্ষেত্রে অল্প সংখ্যক গবেষণা করা হয়েছে। এই গবেষণার উদ্দেশ্য হল খুব অল্প বয়সে খাবার পানিতে আর্সেনিক দোষণের ফলে ১০ - ২০ বৎসর বয়সের মানুষের ফুসফুস, মুত্রগ্রন্থি, রক্তচাপ ও রক্তের চিনির উপর কিভাবে প্রভাব ফেলে তা দেখা।

নির্বাচনের কারণ:

পূর্বের আর্সেনিক গবেষণায় আমরা ৬০০ বাচ্চার উপর আর্সেনিকজনিত চামরার ক্ষত, ফুসফুসের কার্যকারিতা সহ দীর্ঘমেয়াদী স্বাস-প্রস্বাসজনিত রোগের লক্ষণ এবং তাদের খাবার পানিতে আর্সেনিকের মাত্রা নির্ধরণ করেছি। উক্ত গবেষণার তথ্য ব্যবহার করে আমরা দুই দল বাচ্চা বেছে নিয়েছি। আর্সেনিকের মাত্রা বেশী ছিল এমন ৩০০ জন বাচ্চা থেকে আমরা এমন ১০০ জন বাচ্চা নির্ধারণ করব যাদের মাযের পেটে থাকা কালীন অবস্থায় এবং অল্প বয়সের সময় সবচেয়ে উচু মাত্রায় আর্সেনিক ছিল। লিঙ্গ ও বয়স অনুরূপ হওয়া আরো ১০০ বাচ্চা যাদের আর্সেনিকের মাত্রা প্রতি লিটারে ১০ মাইক্রোগ্রাম এর কম ছিল তাদের থেকে নৈর্ব্যক্তিক উপায়ে (লটারী করে) নির্ধারণ করব। সমস্ত অংশগ্রহণকারীদের বয়স ১০ - ২০ বৎসরের মধ্যে হতে হবে। আপনি যেকোনো এক দলে এই বয়স সীমার মাঝে আছেন।

অংশগ্রহণকারীর নিকট থেকে প্রত্যাশা:

আপনি যদি আমাদের এ গবেষণায় অংশগ্রহণ করতে রাজি হন তবে আমাদের মাঠ গবেষণা কর্মী আপনাকে আপনার খাবার পানির উৎস সমুহ, খাদ্য গ্রহণের ইতিহাস, আর্থসামাজিক অবস্থা ইত্যাদি বিষয়ের উপর কিছু প্রশ্ন জিজ্ঞাসা করবেন। এটা করতে এক ঘন্টার মত সময় লাগতে পারে। আপনাকে প্রায় এক ঘন্টা ৩০ মিনিটের জন্য মতলব হাসপাতালে আসতে হবে। হাসপাতালে একজন প্রশিক্ষণপ্রাপ্ত ডাক্তার আপনার স্বাস্থ্য সম্পর্কে ইতিহাস সংগ্রহ এবং রক্তচাপ মাপা ও ফুসফুসের কার্যকারিতা সহ শারীরিক পরীক্ষা করবেন। ফুসফুসের কার্য্যকারিতা পরীক্ষা করার সময় পূর্বের মত আপনাকে একটি নলের মধ্যে খুব জোরে ফুঁ দিতে হবে যেমন করে বেলুন ফুলানো হয়। আর্সেনিকের জন্য চামড়ায় কোনো পরিবর্তন হয়েছে কিনা তাও পরীক্ষা করা হবে। যদি চামড়ায় সন্দেহজনক কোনো পরিবর্তন দেখা যায় তাহলে ঐস্থানের চামড়ার ছবি তোলা হবে যাতে অন্যান্য ডাক্তারগণ দেখতে পারে। জীবানু না ছড়ানোর ব্যাপারে সমস্ত সতর্কতামূলক ব্যবস্থা গ্রহণ পূর্বক একজন ডাক্তার বা নার্স আপনার রক্তের অভুক্ত অবস্থায় চিনির পরিমান, রক্তে সিসি ১৬ (ফুসফুসের কোষের বিষাক্ততা বুঝার নির্দেশক) পরিমাপ করার জন্য আপনার শরীরের শিরা থেকে ৫ মিঃ লিঃ (১ চামচ) পরিমান রক্ত সংগ্রহ করবেন। একজন প্রশিক্ষণ প্রাপ্ত ডাক্তার বা নার্স মুখের কোষ (Buccal cells) সংগ্রহ করার জন্য একটি মুখের সোয়াব (cyto brush) দ্বারা আপনার দুই গালের ভিতরের দিক থেকে মুখের লালা সংগ্রহ করবেন। আর্সেনিকের পরিমাণ মাপার জন্য এবং মুত্রথলীর কোষ (urothelial cell) সংগ্রহের জন্য আমরা আপনাকে ৬০ মিঃ লিঃ প্রস্রাব দেবার জন্য অনুরোধ করব। মুখের কোষ (Buccal cells) এবং মুত্রথলীরকোষ (urothelial cell) উভয়ই DNA বা বংশানুগতি নির্ধারনের ক্ষেত্রে এই পর্য়ন্ত কোনো প্রকার পরিবর্তন হয়েছে তা পরীক্ষা করার জন্য।

গবেষনার ঝুকি এবং অংশগ্রহনের সুবিধা:

এই গবেষনায় অংশগ্রহনে ঝুকি খুবই সামান্য। একজন অভিজ্ঞ এবং প্রশিক্ষণপ্রাপ্ত চিকিৎসক আপনার শারীরিক পরীক্ষা এবং ফুসফুসের কার্য্যকারিতা পরীক্ষা করবেন। শারীরিক এবং ফুসফুসের কার্য্যকারিতা পরীক্ষায় আপনার কোনো আঘাত লাগবেনা। একজন ডাক্তার দ্বারা আপনার সাধারন স্বাস্থ্য পরীক্ষা আপনার জন্য মঙ্গলজনক হতে পারে। জীবানু না ছড়ানোর ব্যাপারে সতর্কতামূলক ব্যবস্থা গ্রহণ করিয়া রক্ত সংগ্রহ করা হবে এবং এতে আপনি সামান্য ব্যাথা অনুভব করতে পারেন।
এই গবেষনায় অংশ্রগ্রহনের জন্য কোন সরাসরি সুবিধাদি নেই। মতলব সেন্টারে যাতায়াতের সমস্ত খরচ এবং ঐ দিনের মজুরী লোকসান বাবদ ৩০০ টাকা এই গবেষণার পক্ষ থেকে আপনাকে দেয়া হবে। মতলব সেন্টারে অপেক্ষাকালীন সময়ে আপনাকে নাস্ড়া এবং পানীয় দেওয়া হবে। এই গবেষণায় অংশগ্রহণের জন্য আপনাকে একটি ব্যাগে প্রয়োজনীয় শিক্ষামূলক জিনিষপত্র উপহার হিসাবে দেওয়া হবে।

গোপনীয়তা, নামহীনতা এবং বিশ্বস্ড়তা :

আমারা আপনাকে জানাতে চাই যে, আপনার স্বাস্থ্যগত পরীক্ষার ফলাফল সহ আপনার এবং আপনার পরিবার থেকে সংগ্রহ করা কোন তথ্য অন্য কাউকে দেওয়া হবে না। শুধূ মাত্র এ গবেষনার গবেষকগণ এবং নীতি পর্যালোচনা কমিটি (ই আর সি) এসব তথ্য দেখতে পারবেন। এই গবেষণায় কোন কাগজ-পত্রে আপনার নাম থাকবে না । প্রত্যেক ব্যক্তির জন্য একটি সাংকেতিক নম্বর দেয়া হবে এবং নামের বদলে এই সাংকেতিক নম্বর ব্যবহার করা হবে। আপনার নাম অথবা কোনো তথ্য যা দিয়ে আপনাকে সনাক্ত করা যায় তাহা প্রকাশ করা হবে না অথবা অন্য কাউকে দেওয়া হবে না।

ভবিষ্যতে ব্যবহারযোগ্য তথ্যঃ

এ গবেষণায় সংগ্রহ করা তথ্য পানিতে আর্সেনিক দূষনের দ্বারা স্বাস্থ্যের উপর প্রভাব সম্পর্কিত গবেষনার কাজে ভবিষ্যতে ব্যবহার করা যেতে পারে। তবে তখনো আপনাকে সনাক্ত করা যায় এমন কোন তথ্য অন্য কাউকে দেওয়া হবে না।

অংশগ্রহণ না করার এবং প্রত্যাহার করার অধিকারঃ

এই গবেষনায় অংশগ্রহন আপনার স্বেচ্ছাধীন। এটার অর্থ এই যে আপনি এই গবেষণায় অংশগ্রহণ নাও করতে পারেন। আপনি যেকোনো সময় এই গবেষনায় অংশ গ্রহণ থেকে বিরত থাকতে পারেন এমনকি যদিও আপনি বলে ছিলেন যে আপনি এটাতে অংশগ্রহন করতে চান।আপনি যাই সিদ্ধান্ত নেন না কেন আপনি এবং আপনার পরিবারের অন্য সদস্যগন আই সি ডি ডি আর বি চিকিৎসা কেন্দ্রগুলো থেকে একই মানের সেবা পাবেন। আপনি কোন অস্বস্ড়ি বোধ করেন এমন কোনো প্রশ্নের উত্তর আপনাকে দিতে হবে না। আপনি এই গবেষনার ব্যাপারে যে কোন প্রশ্ন করতে পারেন এবং আমরা **খুশি মনে** এর উত্তর দেব। এই গবেষণা প্রসঙ্গে আপনি যদি আরও কিছু জানতে চান তাহলে ডাঃ মোঃ ইউনুস, এই গবেষণা কাজের প্রধান গবেষক (মোবাইল নম্বর ০১৭১৩০৯৩৮৭২) অথবা ডাঃ মোঃ আল্ ফজল খান (মোবাইল নম্বর ০১৭১৩০০২৪৭০২) মতলব হাসপাতাল এর সঙ্গে যোগাযোগ করতে পারেন। আপনি যদি মনে করেন যে আপনার সঙ্গে কোন অন্যায় আচরণ করা হয়েছে অথবা এই গবেষণায় যোগদানের ফলে আপনি আঘাতপ্রাপ্ত হয়েছেন তাহলে আপনি জনাব এস এ সালাম খান, সহকারী সমন্বয়কারী ব্যবস্থাপক, আই সি ডি ডি আর বি প্রাতিষ্ঠানিক পর্যালোচনা বোর্ড এর সঙ্গে যোগাযোগ করতে পারেন। জনাব সালাম খানের সঙ্গে যোগাযোগের নাম্বার হল ০২-৯৮৮৪৯৪ (অফিস) অথবা ০২-৮৮৬০৫২৩-৩২ এক্সটেনশন ৩২০৬ অথবা মোবাইল নং - ০১৭১১৪২৮৯৮৯।

আপনার কি কোন প্রশ্ন আছে?	হ্যাঁ	না
আপনি কি এ গবেষণায় অংশগ্রহণের জন্য সম্মত আছেন?	হ্যাঁ	না

এখন আমরা আপনাকে এ গবেষণায় অংশগ্রহনের জন্য আমন্ত্রণ জানাচ্ছি । আপনি যদি এ গবেষণায় অংশগ্রহণ করতে রাজী থাকেন তাহলে অনুগ্রহপূর্বক নীচে নির্ধারিত স্থানে আপনার স্বাক্ষর অথবা বাম হাতের বৃদ্ধাঙ্গুলির টিপ দিন।

_____ _____

অংশগ্রহনকারীর স্বাক্ষর অথবা বাম হাতের বৃদ্ধাঙ্গুলির টিপ তারিখ

_____ _____

প্রধান গবেষক/প্রতিনিধির স্বাক্ষর তারিখ

(নোট: প্রধান গবেষক এর প্রতিনিধির ক্ষেত্রে পূর্ণাঙ্গ নাম, পদবী এবং স্বাক্ষর উলেণ্ঢখ করতে হবে।)

Appendix-2b: Consent form (Bangla)(Pdf)

আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্র, বাংলাদেশ

লিখিত সম্মতি পত্র (Assent)(১১-১৭ বৎসরের ছেলে/মেয়েদের জন্য)

গবেষণা নম্বর: PR- 12012

গবেষণার শিরোনাম: : গর্ভাবস্থায় এবং খুব অল্প বয়সে আর্সেনিকের শোষন এবং বাচ্চাদের ও কিশোর- কিশোরীদের শ্বাস-প্রশ্বাসজনিত অসুখ, রক্তচাপ, ডাইবেটিস এবং মূত্রাশয়ের কার্যকারিতার উপর প্রভাব।

প্রধান গবেষক: ডাঃ [illegible]

গবেষণার উদ্দেশ্য:

আমাদের পূর্বের আর্সেনিক গবেষণায় অংশগ্রহণ-এর জন্য আপনাকে ধন্যবাদ। এখন আমাদের কাছে বিশ্বাসযোগ্য কিছু তথ্য-প্রমাণ আছে যে, আর্সেনিকযুক্ত পানি পান করলে ফুসফুসের রোগ, উচ্চ রক্তচাপ ও ডায়াবেটিস সহ বিভিন্ন ধরনের দীর্ঘমেয়াদী অসুখ হতে পারে। আর্সেনিকযুক্ত পানি পান করলে বড়দের উপর কি প্রতিক্রিয়া হতে পারে এ নিয়ে অনেক গবেষণা করা হয়েছে কিন্তু অল্প বয়স থেকে আর্সেনিকযুক্ত পানি পান করার ফলে বাচ্চাদের ও কিশোর-কিশোরীদের বিভিন্ন রোগের ঝুঁকি যেমন উচ্চরক্তচাপ, ডায়াবেটিস দীর্ঘমেয়াদী অকার্যকারিতা, ফুসফুসের উপর প্রভাব যেমন ফুসফুসের কার্যকারিতা কমে যাওয়া অথবা শ্বাসপ্রশ্বাসজনিত সমস্যার ক্ষেত্রে অল্প সংখ্যক গবেষণা করা হয়েছে। এই গবেষণার উদ্দেশ্য হল খুব অল্প বয়সে খাবার পানিতে আর্সেনিক দূষণের ফলে ১০ - ২০ বৎসর বয়সের মানুষের হৃদযন্ত্র, মূত্রগ্রন্থি, রক্তচাপ ও রক্তের চিনির উপর কিরূপ প্রভাব ফেলে তা দেখা।

নির্বাচনের কারণ:

পূর্বের আর্সেনিক গবেষণায় আমরা ৫০০ বাচ্চার উপর আর্সেনিকজনিত চামড়ার ক্ষত, ফুসফুসের কার্যকারিতা সহ দীর্ঘমেয়াদী শ্বাস-প্রশ্বাসজনিত রোগের লক্ষণ এবং তাদের খাবার পানিতে আর্সেনিকের মাত্রা নির্ধারণ করেছি। উক্ত গবেষণার তথ্য ব্যবহার করে আমরা দুই দল বাচ্চা বেছে নিয়েছি। আর্সেনিকের মাত্রা বেশী ছিল এমন ৫০০ জন বাচ্চা থেকে কিংবা এমন ১০০ জন বাচ্চা নির্বাচন করব যাদের মায়ের পেটে থাকা কালীন অবস্থায় এবং অল্প বয়সের সময় সবচেয়ে বেশি মাত্রায় আর্সেনিক ছিল। ঠিক এ বয়সে অনুরূপ আরো ১০০ বাচ্চা যাদের আর্সেনিকের মাত্রা প্রতি লিটারে ১০ মাইক্রোগ্রাম এর কম ছিল তাদের থেকে দৈবচয়নিক উপায়ে (লটারী করে) নির্বাচন করব। সকল অংশগ্রহণকারীদের বয়স ১০ - ২০ বৎসরের মধ্যে হতে হবে। আপনি যেকোনো এক দলে এই বয়স সীমার মধ্যে আছেন

অংশগ্রহণকারীর নিকট থেকে প্রত্যাশা:

আপনি যদি আমাদের এ গবেষণায় অংশগ্রহণ করতে রাজি হন তবে আমাদের মাঠ গবেষণা কর্মী আপনাকে আপনার খাবার পানির উৎস সমূহ, খাদ্য গ্রহণের ইতিহাস, আর্থসামাজিক অবস্থা ইত্যাদি বিষয়ের উপর কিছু প্রশ্ন জিজ্ঞাসা করবেন। এটা করতে এক ঘন্টার মত সময় লাগতে পারে। আপনাকে প্রায় এক ঘন্টা ৩০ মিনিটের জন্য মতলব হাসপাতালে আসতে হবে। হাসপাতালে একজন প্রশিক্ষণপ্রাপ্ত ডাক্তার আপনার স্বাস্থ্য সম্পর্কে ইতিহাস সংগ্রহ এবং রক্তচাপ মাপা ও হৃদযন্ত্রের কার্যকারিতা সহ শারীরিক পরীক্ষা করবেন। ফুসফুসের কার্যকারিতা পরীক্ষা করার সময় পূর্বের মত আপনাকে একটি নলের মধ্যে খুব জোরে ফুঁ দিতে হবে যেমন করে মোমবাতি নেভানো হয়। আর্সেনিকের জন্য চামড়ায় কোনো পরিবর্তন হয়েছে কিনা তাও পরীক্ষা করা হবে। যদি কোন সন্দেহজনক কোনো পরিবর্তন দেখা যায় তাহলে প্রয়োজনে চামড়ার ছবি তোলা হবে যাতে অন্যান্য বিশেষজ্ঞগণ দেখতে পারে। জীবাণু না ছড়ানোর ব্যাপারে যথাযথ সতর্কতামূলক ব্যবস্থা গ্রহণ পূর্বক একজন ডাক্তার বা নার্স আপনার রক্তের অভুক্ত অবস্থায় চিনির পরিমান, হিমোগ্লোবিন এ১সি (গ্লাইকোহিমোগ্লোবিন/ডায়াবেটিস নির্ণয়ক) পরিমাপ করার জন্য আপনার শরীরের শিরা থেকে ৫ মিঃ লিঃ (১ চামচ) পরিমান রক্ত সংগ্রহ করবেন। একজন প্রশিক্ষণ প্রাপ্ত ডাক্তার বা নার্স মুখের কোষ (Buccal cells) সংগ্রহ করার জন্য একটি সূক্ষ্ম ব্রাশ (cyto brush) দ্বারা আপনার দুই গালের ভিতরের দিক থেকে মুখের লালা সংগ্রহ করবেন। আর্সেনিকের পরিমাপ করার জন্য এবং মূত্রথলীর কোষ (urothelial cell) সংগ্রহের জন্য আমরা আপনাকে ৩০ মিঃ লিঃ প্রস্রাব দেবার জন্য অনুরোধ করব। মুখের কোষ (Buccal cells) এবং মূত্রথলীর কোষ (urothelial cell) উভয়ই DNA বা বংশগতি নির্ধারণের ক্ষেত্রে এই পর্যন্ত কোনো প্রকার পরিবর্তন হয়েছে তা পরীক্ষা করার জন্য।

গবেষনার ঝুঁকি এবং অংশগ্রহনের সুবিধা:

এই গবেষনায় অংশগ্রহণে ঝুঁকি খুবই সামান্য। একজন অভিজ্ঞ এবং প্রশিক্ষণপ্রাপ্ত চিকিৎসক আপনার শারীরিক পরীক্ষা এবং ফুসফুসের কার্যকারিতা পরীক্ষা করবেন। শারীরিক এবং ফুসফুসের কার্যকারিতা পরীক্ষায় আপনার কোনো আঘাত লাগবেনা। একজন ডাক্তার দ্বারা আপনার সাধারণ স্বাস্থ্য পরীক্ষা আপনার জন্য মঙ্গলজনক হতে পারে। জীবাণু না ছড়ানোর ব্যাপারে সতর্কতামূলক ব্যবস্থা গ্রহণ পূর্বক রক্ত সংগ্রহ করা হবে এবং এতে আপনি সামান্য ব্যথা অনুভব করতে পারেন

আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্র, বাংলাদেশ
লিখিত সম্মতি পত্র (১৮-২০ বৎসরের জন্য)

গবেষণা নম্বর: PR- 12012
গবেষণার শিরোনাম: : গর্ভাবস্থায় এবং খুব অল্প বয়সে আর্সেনিকের শোষণ এবং বাচ্চাদের ও কিশোর- কিশোরীদের শ্বাস-প্রশ্বাসজনিত অসুখ, রক্তচাপ, ডাইবেটিক এবং মূত্রাশয়ের কার্যকারিতার উপর প্রভাব।
প্রধান গবেষক: ডাঃ মোহাম্মদ ইউনূস

গবেষণার উদ্দেশ্য:
আমাদের পূর্বের আর্সেনিক গবেষণায় অংশগ্রহণ-এর জন্য আপনাকে ধন্যবাদ। এখন আমাদের কাছে বিশ্বাসযোগ্য কিছু তথ্য-প্রমাণ আছে যে, আর্সেনিকযুক্ত পানি পান করলে ফুসফুসের রোগ, উচ্চ রক্তচাপ ও ডায়াবেটিস সহ বিভিন্ন ধরনের দীর্ঘমেয়াদী অসুখ হতে পারে। আর্সেনিকযুক্ত পানি পান করলে বড়দের উপর কি প্রতিক্রিয়া হতে পারে তা নিয়ে অনেক গবেষণা করা হয়েছে কিন্তু অল্প বয়স থেকে আর্সেনিকযুক্ত পানি পান করার ফলে বাচ্চাদের ও কিশোর-কিশোরীদের বিভিন্ন রোগের ঝুঁকি যেমন উচ্চরক্তচাপ, হৃৎপিণ্ড দীর্ঘমেয়াদী অকার্যকারীতা, ফুসফুসের উপর প্রভাব যেমন ফুসফুসের কার্যকারিতা কমে যাওয়া অথবা শ্বাসপ্রশ্বাসজনিত লক্ষণের উপর অল্প সংখ্যক গবেষণা করা হয়েছে। এই গবেষণার উদ্দেশ্য হল খুব অল্প বয়সে খাবার পানিতে আর্সেনিক শোষণের ফলে ১৮ - ২০ বছর বয়সের মানুষের হৃদযন্ত্র, মূত্রথলি, রক্তচাপ ও রক্তের চিনির উপর কিভাবে প্রভাব ফেলে তা দেখা।

নির্বাচনের কারণ:
পূর্বের আর্সেনিক গবেষণায় আমরা ৬০০ বাচ্চার উপর আর্সেনিকজনিত চর্মরোগ ক্ষত, হৃদযন্ত্রের কার্যকারিতা সহ দীর্ঘমেয়াদী শ্বাস-প্রশ্বাসজনিত রোগের লক্ষণ এবং তাদের খাবার পানিতে আর্সেনিকের মাত্রা নির্ণয় করেছি। উক্ত গবেষণার তথ্য ব্যবহার করে আমরা দুই দল বাচ্চা বেছে নিয়েছি। আর্সেনিকের মাত্রা বেশী ছিল এমন ৬০০ জন বাচ্চা থেকে আমরা এমন ১০০ জন বাচ্চা নির্ধারণ করব যাদের মায়ের পেটে থাকা কালীন অবস্থায় এবং অল্প বয়সের সময় সবচেয়ে উচ্চ মাত্রায় আর্সেনিক ছিল। লিঙ্গ ও বয়স অনুরূপ এমন আরো ১০০ বাচ্চা যাদের আর্সেনিকের মাত্রা প্রতি লিটারে ১০ মাইক্রোগ্রাম এর কম ছিল তাদের থেকে দৈবচয়িক উপায়ে (লটারী করে) নির্বাচন করব। সকল অংশগ্রহণকারীদের বয়স ১৮ - ২০ বৎসরের মধ্যে হতে হবে। আপনি যেকোনো এক দলে এবং বয়সে সীমার মধ্যে আছেন

অংশগ্রহণকারীর নিকট থেকে প্রত্যাশা:
আপনি যদি আমাদের এ গবেষণায় অংশগ্রহণ করতে রাজি হন তবে আমাদের মাঠ গবেষণা কর্মী আপনাকে আপনার খাবার পানির উৎস সমূহ, খাদ্য গ্রহণের ইতিহাস, আর্থসামাজিক অবস্থা ইত্যাদি বিষয়ের উপর কিছু প্রশ্ন জিজ্ঞাসা করবেন। এটা করতে এক ঘন্টার মত সময় লাগতে পারে। আপনাকে প্রায় এক ঘন্টা ৩০ মিনিটের জন্য মতলব হাসপাতালে আসতে হবে। হাসপাতালে একজন প্রশিক্ষণপ্রাপ্ত ডাক্তার আপনার স্বাস্থ্য সম্পর্কে ইতিহাস সংগ্রহ এবং রক্তচাপ মাপা ও হৃদযন্ত্রের কার্যকারিতা সহ শারীরিক পরীক্ষা করবেন। ফুসফুসের কার্যকারিতা পরীক্ষা করার সময় পূর্বের মত আপনাকে একটি নলের মধ্যে খুব জোরে ফুঁ দিতে হবে যেমন করে বেলুন ফুলানো হয়। আর্সেনিকের জন্য চামড়ায় কোনো পরিবর্তন হয়েছে কিনা তাও পরীক্ষা করা হবে। যদি চামড়ায় উল্লেখযোগ্য কোনো পরিবর্তন দেখা যায় তাহলে ঐ স্থানের চামড়ার ছবি তোলা হবে যাতে অন্যান্য ও কারণগুলি দেখতে পারে। জীবাণু না ছড়ানোর ব্যাপারে সকল সতর্কতামূলক ব্যবস্থা গ্রহণ পূর্বক একজন ডাক্তার বা নার্স আপনার রক্তের অভুক্ত অবস্থায় চিনির পরিমাণ, রক্তে সিসি ১৬ (ফুসফুসের কোষের বিষাক্ততা বুঝার নির্দেশক) পরিমাপ করার জন্য আপনার শরীরের শিরা থেকে ৫ মিলি (১ চামচ) পরিমাণ রক্ত সংগ্রহ করবেন। একজন প্রশিক্ষণ প্রাপ্ত ডাক্তার বা নার্স মুখের কোষ (Buccal cells) সংগ্রহ করার জন্য একটি ছোট্ট ব্রাশ (cyto brush) দ্বারা আপনার দুই গালের ভিতরের দিক থেকে মুখের লালা সংগ্রহ করবেন। আর্সেনিকের পরিমাণ মাপার জন্য এবং মূত্রনালীর কোষ (urothelial cell) সংগ্রহের জন্য আমরা আপনাকে ৩০ মিলি লিটার প্রস্রাব দেবার জন্য অনুরোধ করব। মুখের কোষ (Buccal cells) এবং মূত্রনালীর কোষ (urothelial cell) উভয়ই DNA বা বংশগতি নির্ধারণের ক্ষেত্রে এই খাবার কোনো প্রকার পরিবর্তন হয়েছে তা পরীক্ষা করার জন্য

গবেষনার ঝুঁকি এবং অংশগ্রহনের সুবিধা:
এই গবেষনায় অংশগ্রহণে ঝুঁকি খুবই সামান্য। একজন অভিজ্ঞ এবং প্রশিক্ষণপ্রাপ্ত চিকিৎসক আপনার শারীরিক পরীক্ষা এবং ফুসফুসের কার্যকারিতা পরীক্ষা করবেন। শারীরিক এবং হৃদযন্ত্রের কার্যকারিতা পরীক্ষায় আপনার কোনো আঘাত লাগবে না। একজন ডাক্তার দ্বারা আপনার সাধারণ স্বাস্থ্য পরীক্ষা আপনার জন্য মঙ্গলজনক হতে পারে। জীবাণু না ছড়ানোর ব্যাপারে সতর্কতামূলক ব্যবস্থা গ্রহণ পূর্বক রক্ত সংগ্রহ করা হবে এবং এতে আপনি সামান্য ব্যথা অনুভব করতে পারেন

Appendix 3a: Parental main quenstionnair

PARENTAL MAIN QUESTIONNAIRE

CID: |___|___|___|___|___|___|___|___|___|___|

RID: |___|___|___|___|___|___|___|___|___|___|

Starting Time: |___|___| . |___|___| AM/PM **Date:** |__|__|__|__|__|__|
DD/MM/YY

Respondent will be the primary caretaker (usually the mother) of the participant. For questions with [child's name], please insert the participant's name when asking the question.

Respondent's relationship to participant:_____

এখন আমি কিছু সাধারন প্রশ্ন জিজ্ঞাসা করব---------- (সন্তানের নাম)'র আর আপনার পরিবার সম্পর্কে।
I am going to start by asking you some general questions about [child's name] and your family.

1. _____ (সন্তানের নাম) বয়স কত? / What is [child's name]'s age? |___|___| Years

(If the parent is not sure about participant's birth date, ask the following questions.)

2. _____ (সন্তানের নাম) কোন ক্লাসে পড়ে? |___|___|
2. Which grade school is [child's name] in currently?
("**NA**" means the child does not goes to school currently)

3. বড়, ছোট মিলিয়ে আপনার পরিবারে মোট কতজন একই ঘরে বাস করেন? |___|___|
3. How many family members live in your current house (include both children and adults)?

RESIDENCE HISTORY

(Keep inserting the years, house and water source numbers as you ask the following questions in the time log boxes on the loose sheet. Staple the sheet at the end of the interview. This is to work out the historical drinking and cooking water exposure throughout the participant's life time. The detailed questions are on the following pages.)

1. _____ (সন্তানের নাম) কোন সালে জন্মেছিল?

(Insert the year on the line above, at the spot noted by YOB of the child)

2. (সন্তানের নাম) জন্মাবার পর থেকে কি এই বাড়িতেই আছেন?
2. Have you been staying in the same house since our last visit? ☐ Yes → Go to page 2.
☐ / No

3. _____ ((সন্তানের নাম) জন্মাবার পর কতগুলি বাড়িতে থেকেছেন? (৬ মাস অথবা অধিক)
|___|___|
3. How many houses have you lived in since our last visit(6 months or more)?

RESIDENTIAL WATER HISTORY - CURRENT HOUSE, CURRENT SOURCE
SECTION H1T1

1a কোন সালে এই বাড়িতে প্রথম আসা হয়েছিল? |___|___|___|___|

1a . In what year did you move into your current house (house #1)?

1b. _____ (সন্তানের নাম) বয়স তখন কতছিল? |___|___| বছর

(How old was [child's name] then?)
("xx" means the child was not born)

1c (সন্তানের নাম)এই বাড়িতে থাকাকালীন পানির জায়গার কোন পরিবর্তন হয়েছে?

1c. Was there any change in your water source while you lived in your current house? ☐ Yes

☐ No

→ Go to Q 1e

1d. কতবার পানির জায়গা পরিবর্তন হয়েছে? (How many changes were there in your water source?) |___|___|

Here 00 means no change in water source. 01 means she had two water sources (T1 & T2).

1e. আপনি কি এখন খাওয়া ও রান্নার জন্য একটি নাকি একাধিক জায়গা থেকে পানি আনেন?

1e. Do you currently get water for drinking and cooking from just one source or more than one source (check)?

☐ মাত্র একটি (Just one)

☐ একের বেশী (More than one) → Go to Q.2

1f. পানির জায়গা কি? কোথা থেকে পানি আনেন? (কল না পাত কুয়ো না পুকুর না টাইম কল?)

1f. i) What is the water source?

_____

(Probe: What is it? For example, which tubewell, pond, supply water? What is the location?)

ii) Sample collected Y / N iii) Sample code number_____

iv) Location or TW ID # .....

.

v) Name of the tubewell owner .....

2. কতগুলো জায়গা থেকে এখন পানি আনেন? (How many sources now?) |___|___|

3. সব পানির জায়গাগুলো বলুন আর কোথা থেকে কতভাগ পানি খাওয়া ও রান্নার জন্য নেওয়া হয় তার পরিমাণ বলুন?

. Please tell me the location of all your water sources, and how much of your drinking and cooking water you obtain from each of these sources.

(Note: Identify each source so that it can be located. Write in the location. Circle the proportion for each source and make sure the proportions add to one for all the sources combined.)

3a. i) আপনাদের বেশিরভাগ সময় কোথা থেকে খাওয়া ও রান্নার জন্য পানি আনেন?

i) What is the primary source of drinking and cooking water? _____

ii) ঐখান থেকে মেট কতভাগ পানি ব্যবহার করেন?

ii) What is the proportion of water you use from this source? <1/4, 1/4, 1/2, 3/4, >3/4

ii) Sample collected Y / N iii) Sample code number_____

iv) Location or TW ID # .....

v) Name of the tube well owner .....

3b. i) আপনাদের দ্বিতীয় পানির জায়গা কি?
i) What is the second source of drinking and cooking water? _____
ii) ঐখান থেকে কি পরিমাণ পানি ব্যবহার করেন?
ii) What is the proportion of water you use from this source? <1/4, 1/4, 1/2, 3/4, >3/4

ii) Sample collected Y / N iii) Sample code number_____

iv) Location or TW ID # .....

v) Name of the tubewell owner .....

3c. i) আপনাদের পানির তৃতীয় জায়গা কি?
i) What is the third source of drinking and cooking water? _____
ii) ঐখান থেকে কি পরিমাণ পানি ব্যবহার করেন?
ii) What is the proportion of water you use from this source? <1/4, 1/4, 1/2, 3/4, >3/4

ii) Sample collected Y / N iii) Sample code number_____

iv) Location or TW ID # .....

v) Name of the tubewell owner .....

3d. i) আপনাদের চতুথ পানির্ জায়গা কি?
i) What is the fourth source of drinking and cooking water? _____
ii) ঐখান থেকে কি ভাগ পরিমাণ পানি ব্যাবহার করেন?
ii) What is the proportion of water you use from this source? <1/4, 1/4, 1/2, 3/4, >3/4

ii) Sample collected Y / N iii) Sample code number_____

iv) Location or TW ID # .....

v) Name of the tubewell owner .....

যদি বর্তমান বাড়িতে থাকা অবস্থায় পানির উৎসের পরিবর্তন হয় তাহলে ৪-৫ নং পাতা পূরণ করুন। বর্তমান বাড়িতে থাকা অবস্থায় যদি আরো পানির উৎসের পরিবর্তনের হয় তাহলে অতিরিক্ত ৪-৫ নং পাতা সংযুক্ত করে পূরণ করুন।

যদি অংশগ্রহণকারী জন্মের পর থেকে শুধু মাত্র বর্তমান বাড়িতেই বাস করে থাকে, তাহলে ১২ নং পাতা পূরণ করুন।
যদি অংশগ্রহণকারী বর্তমান বাড়ির আগে অন্য বাড়ীতে বাস করে থাকে, তাহলে ৬-৭ নং পাতা পূরণ করুন। অংশগ্রহণকারীর প্রতিবার বাড়ি পরিবর্তনের জন্য অতিরিক্ত ৮-৯ নং পাতা সংযুক্ত করুন। (যেমন- **H3T1, H4T1……)**

If there was a CHANGE in the water source at the CURRENT HOUSE while the participant lived in current house, fill out pages 4-5. Insert a copy of pages 4-5 for EACH ADDITIONAL CHANGE in water source at the CURRENT HOUSE, going back in time. Fill in the water source number at the top of the page, H1T2, H1T3 etc.

If participant has ONLY LIVED IN THE CURRENT HOUSE since he/she was born, go to page 12.

If participant has lived in a PREVIOUS HOUSE before the current house, fill out pages 6-7. If there was a CHANGE in the water source at the PREVIOUS HOUSE while the participant lived in current house, fill out pages 4-5.Insert a copy of pages 8-9 for EACH ADDITIONAL HOUSE the participant has lived in, going back in time. Fill in the house number at the top of the page, H3T1, H4T1 etc.

RESIDENTIAL WATER HISTORY – CURRENT HOUSE, CHANGE IN WATER SOURCESECTION

H1T□

Note: Insert a copy of pages 4-5 for EACH CHANGE in water source at CURRENT HOUSE, going back in time. Fill in source number at the top of the page, H1T2, H1T3 etc.

1a. কত বৎসর আগে এই H1T □খাওয়া ও রান্নার পানির জায়গা বদল করেছিলেন?

1a. How many years ago did the change in drinking and cooking water source occur at your current house?

|___|___| বছর

1b. কোন সালে এই খাওয়া ও রান্নার পানির জায়গা বদল করেছিল?

1b. In what year did this change in drinking and cooking water source occur?

|___|___|___|___|

1c. যখন পানির জায়গা বদল হয়েছিল তখন ---------- (সন্তানের নাম)'র বয়স কত ছিল?

1c. How old was [child's name] when the change occurred?

|___|___| বছর

(Note: If there is a discrepancy in Q1, please clarify it with the interviewee.)

2a. _____*(Answer 1a)* বছর আগে আপনারা যখন খাওয়া ও রান্নার জন্য পানির জায়গা বদল করেছিলেন তখন কি একটা জায়গা থেকে পানি আনতেন না একাধিক জায়গা থেকে পানি আনতেন?

2a. Before the change, did you get water for drinking and cooking from just one source or more than one source (check)? □ মাত্র একটি (Just one) □ একের বেশী (More than one) → Go to Q.3

2b. i) খাওয়া ও রান্নার জন্য পানির জায়গা কি ছিল? কোথা থেকে পানি খেত? (কল না পাত কুয়ো না পুকুর না টাইম কল)

2b. i) What was the water source for drinking and cooking?

_____

(Probe: What is it? For example, which tubewell, pond, supply water? What is the location?)

ii) Sample collected Y / N iii) Sample code number_____

iv) Location or TW ID # .....

.

v) Name of the tubewell owner .....

3. কতগুলো জায়গা থেকে খাওয়া ও রান্নার জন্য পানি আনতেন? (How many sources?) |___|___|

4. ঐসব পানির জায়গাগুলো কোথায় ছিল আর কোথা থেকে কত ভাগ পানি ব্যবহার করা হত তার পরিমাণ বলুন?

4. Please tell me the location of all your water sources, and how much of your drinking and cooking water you obtained from each of these sources.

(Note: Identify each source so that it can be located. Write in the location. Circle the proportion for each source and make sure the proportions add to one for all the sources combined.)

4a. i) আপনাদের বেশীরভাগ সময় কোথা থেকে খাওয়া ও রান্নার জন্য পানি আনতেন?

i) What was the primary source of drinking and cooking water?

_____

ii) ঐখান থেকে মোট কতভাগ পানি ব্যবহার করা হত?

ii) What was the proportion of water you use from this source? <1/4, 1/4, 1/2, 3/4, >3/4

ii) Sample collected Y / N iii) Sample code number_____

iv) Location or TW ID # .....

v) Name of the tubewell owner .....

4b. i) আপনাদের দ্বিতীয় পানির জায়গা কি ছিল?

i) What was the second source of drinking and cooking water?

_____

ii) ঐখান থেকে মোট কত ভাগ পানি ব্যবহার করা হত?

ii) What was the proportion of water you use from this source? <1/4, 1/4, 1/2, 3/4, >3/4

ii) Sample collected Y / N iii) Sample code number_____

iv) Location or TW ID # .....

v) Name of the tubewell owner .....

4c. i) আপনাদের পানির জায়গা কি ছিল?

i) What was the third source of drinking and cooking water?

_____

ii) ঐখান থেকে মোট কতভাগ পানি ব্যবহার করা হত?

ii) What was the proportion of water you use from this source? <1/4, 1/4, 1/2, 3/4, >3/4

ii) Sample collected Y / N iii) Sample code number_____

iv) Location or TW ID # .....

v) Name of the tubewell owner .....

4d. i) আপনাদের পানির জায়গা কি ছিল?

i) What was the fourth source of drinking and cooking water?

_____

ii) ঐখান থেকে মোট কতভাগ পানি ব্যবহার করা হত?

ii) What was the proportion of water you use from this source? <1/4, 1/4, 1/2, 3/4, >3/4

ii) Sample collected Y / N iii) Sample code number_____

iv) Location or TW ID # .....

v) Name of the tubewell owner .....

বর্তমান বাড়িতে থাকা অবস্থায় যদি আরো পানির উৎসের পরিবর্তনের হয় তাহলে অতিরিক্ত ৪-৫ নং পাতা সংযুক্ত করে পূরণ করুন। (যেমন-HIT3, HIT4)

যদি অংশগ্রহণকারী জন্মের পর থেকে শুধুমাত্র বর্তমান বাড়িতেই বাস করে থাকে, তাহলে ১২ নং পাতা পূরণ করুন।

যদি অংশগ্রহণকারী বর্তমান বাড়ির আগে অন্য বাড়িতে বাস করে থাকে , তাহলে ৬-৭ নং পাতা পূরণ করুন। অংশগ্রহণকারী প্রতিবার বাড়ি পরিবর্তনের জন্য অতিরিক্ত ৮-৯ নং পাতা সংযুক্ত করুন। (যেমন-HIT3, HIT4……)

Insert a copy of pages 4-5 for EACH ADDITIONAL CHANGE in water source at the CURRENT HOUSE, going back in time. Fill in the water source number at the top of the page, H1T3, H1T4 etc.

If participant has ONLY LIVED IN THE CURRENT HOUSE since he/she was born, go to page 12.

If participant has lived in a PREVIOUS HOUSE before the current house, fill out pages 6-7. For EACH ADDITIONAL CHANGE in water source at the PREVIOUS HOUSE fill out pages 8-9. Insert a copy of pages 8-9 for EACH ADDITIONAL CHANGE in water source at the PREVIOUS HOUSE, going back in time. Fill in the water source number at the top of the page, H2T3, H2T4 etc. For EACH ADDITIONAL HOUSE the participant has lived in, going back in time. Fill in the house number at the top of the page, H3T1, H4T1 etc.

ESIDENTIAL WATER HISTORY – PREVIOUS HOUSE, LAST SOURCE

SECTION H2T1

1a. বর্তমান এই বাড়ির আগে _____ [সন্তানের নাম] কোথায় থাকত?
1a. Before your current house where did [child's name] live? (Check with the record)

_____

1b. কোন সালে এই বাড়িতে (H2) প্রথম আসা হয়েছিল?
1b. In what year did you move into your previous house (house #2)?
|___|___|___|___|

1c এই বাড়িতে থাকাকালীন পানির জায়গার কোন পরিবর্তন হয়েছে?

1c. Was there any change in your water source while you lived in your previous house (house#2)? ☐ হ্যাঁ / Yes

☐ না / No → Go to Q 1e

1d. কতবার পানির জায়গা পরিবর্তন হয়েছে? (How many changes were there in your water source?) |___|___|

1e. _____ [সন্তানের নাম] আগের ঐ বাড়িতে কি একটা না একাধিক জায়গা থেকে পানি খাওয়া ও রান্নার জন্য ব্যবহার করতেন?
1e. At the previous house, did you get water for drinking and cooking from just one source or more than one source (check)?

☐ মাত্র একটি (Just one)

☐ একের বেশী (More than one) → Go to Q.2

1f. কতদিন ধরে ঐ পানি (H2T1) ব্যবহার করেছেন? |___|___| বছর (year) / মাস (month)
1f. How long did you use that water?

1g. i) খাওয়া ও রান্নার পানির জায়গা (H2T1) কি ছিল ও কোথা থেকে পানি আনা হত? (কল না পাত কুয়ো না পুকুর, না টাইম কল?)
1g. i) What was the water source? _____

(Probe: What is it? For example, which tubewell, pond, supply water? What is the location?)

ii) Sample collected Y / N iii) Sample code number_____

iv) Location or TW ID # .....

v) Name of the tubewell owner .....

2. খাওয়া ও রান্নার পানির জায়গা কতগুলো ছিল? (How many sources?) |___|___|

3. সব পানির জায়গা গুলো কোথায় ছিল বলুন আর কোথা থেকে কত ভাগ পানি আনা হত তার পরিমাণ বলুন?
3. Please tell me the location of all your water sources, and how much of your drinking and cooking water you obtained from each of these sources.
(Note: Identify each source so that it can be located. Write in the location. Circle the proportion for each source and make sure the proportions add to one for all the sources combined.)

3a. i) ঐ আগের বাড়িতে আপনাদের বেশীরভাগ সময় কোথা থেকে *খাওয়া ও রান্নার জন্য* পানি আনতেন ?
i) What was the primarysource of drinking and cooking water? _____
ii) ঐখান থেকে মোট কত ভাগ পানি ব্যবহার করা হত?
ii) What was the proportion of water you use from this source? <1/4, 1/4, 1/2, 3/4, >3/4

ii) Sample collected Y / N iii) Sample code number_____

iv) Location or TW ID # .....

v) Name of the tubewell owner .....

3b. i) তখন ওখানকার দ্বিতীয় পানির জায়গা কি ছিল?
i) What was the second source of drinking and cooking water? _____
ii) ঐখান থেকে মোট কত ভাগ পানি ব্যবহার করা হত?
ii) What was the proportion of water you use from this source? <1/4, 1/4, 1/2, 3/4, >3/4

ii) Sample collected Y / N iii) Sample code ber_____

iv) Location or TW ID # .....

v) Name of the tubewell owner .....

3c. i) তখন ওখানকার আপনাদের পানির তৃতীয় জায়গা কি ছিল?
i) What was the third source of drinking and cooking water?
_____
ii) ঐখান থেকে মোট কত ভাগ পানি ব্যবহার করা হত?
ii) What was the proportion of water you use from this source? <1/4, 1/4, 1/2, 3/4, >3/4

ii) Sample collected Y / N iii) Sample code number_____

iv) Location or TW ID #.....

v) Name of the tubewell owner .....

3d. i) তখন ওখানকার আপনাদের চর্তুথ পানির জায়গা কি ছিল ?
i) What was the fourth source of drinking and cooking water?
_____
ii) ঐখান থেকে মোট কত ভাগ পানি ব্যবহার করা হত?
ii) What was the proportion of water you use from this source? <1/4, 1/4, 1/2, 3/4, >3/4

ii) Sample collected Y / N iii) Sample code number_____

iv) Location or TW ID # .....

v) Name of the tube well owner .....

যদি বর্তমান বাড়িতে থাকা অবস্থায় পানির উৎসের পরিবর্তন হয় তাহলে ৪-৫ নং পাতা পূরণ করুন। বর্তমান বাড়িতে থাকা অবস্থায় যদি আরো পানির উৎসের পরিবর্তনের হয় তাহলে অতিরিক্ত ৪-৫ নং পাতা সংযুক্ত করে পূরণ করুন। (যেমন- H1T2, H1T3.....)

যদি অংশগ্রহনকারী বর্তমান বাড়ির আগে অন্য বাড়ীতে বাস করে থাকে, তাহলে ৬-৭ নং পাতা পূরণ করুন। অংশগ্রহনকারীর প্রতিবার বাড়ি পরিবর্তনের জন্য অতিরিক্ত ৮-৯ নং পাতা সংযুক্ত করুন। (যেমন- H3T1, H4T1.....)

If there was a CHANGE in the water source at the PREVIOUS HOUSE while the participant lived in previous house, fill out pages 8-9. Insert a copy of pages 8-9 for EACH ADDITIONAL CHANGE in water source at the PREVIOUS HOUSE, going back in time. Fill in the water source number at the top of the page, H2T2, H2T3 etc.

If participant has lived in another house before the PREVIOUS HOUSE, fill out pages 10-11, Insert a copy of pages 10-11 for EACH ADDITIONAL HOUSE the participant has lived in, going back in time. Fill in the house number at the top of the page, H4T1, H5T1 etc.

RESIDENTIAL WATER HISTORY – PREVIOUS HOUSE, CHANGE IN WATER SOURCE SECTION

H2T□

If there was a CHANGE in the water source at the PREVIOUS HOUSE while the participant lived in previous house, fill out pages 8-9. Insert a copy of pages 8-9 for EACH ADDITIONAL CHANGE in water source at the PREVIOUS HOUSE, going back in time. Fill in the water source number at the top of the page, H2T2, H3T3 etc.

1a. কত বৎসর আগে এই H1T □খাওয়া ও রান্নার পানির জায়গা বদল করেছিলেন?

1a. How many years ago did the change in drinking and cooking water source occur at your current house?

|___|___| বছর

1b. কোন সালে এই খাওয়া ও রান্নার পানির জায়গা বদল করেছিল?

1b. In what year did this change in drinking and cooking water source occur?

|___|___|___|___|

1c. যখন পানির জায়গা বদল হয়েছিল তখন ---------- (সন্তানের নাম)'র বয়স কত ছিল?

1c. How old was [child's name] when the change occurred?

|___|___| বছর

(Note: If there is a discrepancy in Q1, please clarify it with the interviewee.)

2a. _____*(Answer 1a)* বছর আগে আপনারা যখন খাওয়া ও রান্নার জন্য পানির জায়গা বদল করেছিলেন তখন কি একটা জায়গা থেকে পানি আনতেন না একাধিক জায়গা থেকে পানি আনতেন?

2a. Before the change, did you get water for drinking and cooking from just one source or more than one source (check)?

☐ মাত্র একটি (Just one) ☐ একের বেশী (More than one) → Go to Q.3

2b. i) খাওয়া ও রান্নার জন্য পানির জায়গা কি ছিল? কোথা থেকে পানি খেত? (কল না পাত কুয়ো না পুকুর না টাইম কল)

2b. i) What was the water source for drinking and cooking?

_____

(Probe: What is it? For example, which tubewell, pond, supply water? What is the location?)

ii) Sample collected Y / N iii) Sample code number_____

iv) Location or TW ID # ……

.

v) Name of the tubewell owner ……

3. কতগুলো জায়গা থেকে খাওয়া ও রান্নার জন্য পানি আনতেন? (How many sources?) |___|___|

4. ঐসব পানির জায়গাগুলো কোথায় ছিল আর কোথা থেকে কত ভাগ পানি ব্যবহার করা হত তার পরিমাণ বলুন?

4. Please tell me the location of all your water sources, and how much of your drinking and cooking water you obtained from each of these sources.

(Note: Identify each source so that it can be located. Write in the location. Circle the proportion for each source and make sure the proportions add to one for all the sources combined.)

4a. i) আপনাদের বেশীরভাগ সময় কোথা থেকে খাওয়া ও রান্নার জন্য পানি আনতেন?

i) What was the primary source of drinking and cooking water?

_____

ii) ঐখান থেকে মোট কতভাগ পানি ব্যবহার করা হত?

ii) What was the proportion of water you use from this source? <1/4, 1/4, 1/2, 3/4, >3/4

ii) Sample collected Y / N iii) Sample code number_____

iv) Location or TW ID # ……

v) Name of the tubewell owner ……

3b. i) তখন ওখানকার দ্বিতীয় পানির জায়গা কি ছিল?

i) What was the second source of drinking and cooking water? _____

ii) ঐখান থেকে মোট কত ভাগ পানি ব্যবহার করা হত?

ii) What was the proportion of water you use from this source? <1/4, 1/4, 1/2, 3/4, >3/4

ii) Sample collected Y / N iii) Sample code ber_____

iv) Location or TW ID # ……

v) Name of the tubewell owner ……

3c. i) তখন ওখানকার আপনাদের পানির তৃতীয় জায়গা কি ছিল?

i) What was the third source of drinking and cooking water?

_____

ii) ঐখান থেকে মোট কত ভাগ পানি ব্যবহার করা হত?

ii) What was the proportion of water you use from this source? <1/4, 1/4, 1/2, 3/4, >3/4

ii) Sample collected Y / N iii) Sample code number_____

iv) Location or TW ID #……

v) Name of the tubewell owner ……

3d. i) তখন ওখানকার আপনাদের চর্তুথ পানির জায়গা কি ছিল ?

i) What was the fourth source of drinking and cooking water?

_____

ii) ঐখান থেকে মোট কত ভাগ পানি ব্যবহার করা হত?

ii) What was the proportion of water you use from this source? <1/4, 1/4, 1/2, 3/4, >3/4

ii) Sample collected Y / N iii) Sample code number_____

iv) Location or TW ID # ……

v) Name of the tube well owner ……

যদি অংশগ্রহনকারী বর্তমান বাড়ির আগে অন্য বাড়ীতে বাস করে থাকে, তাহলে ৬-৭ নং পাতা পূরণ করুন। অংশগ্রহনকারীর প্রতিবার বাড়ি পরিবর্তনের জন্য অতিরিক্ত ৮-৯ নং পাতা সংযুক্ত করুন। (যেমন- H3T1, H4T1……)

If participant has lived in another house before the PREVIOUS HOUSE fill out pages 10-11, Insert a copy of pages 10-11 for EACH ADDITIONAL HOUSE the participant has lived in, going back in time. Fill in the house number at the top of the page, H4T1, H5T1 etc.

RESIDENTIAL WATER HISTORY – ANY PREVIOUS HOUSE, LAST SOURCE

SECTION H□T1

Note: Insert a copy of pages 10-11 for EACH ADDITIONAL HOUSE the participant has lived in, going back in time. Fill in the house number at the top of the page, H3T1, H4T1 etc.

1a. আগের বাড়ির আগে _____ [সন্তানের নাম] কোথায় থাকত?
1a Before the last house where did [child's name] live? (Check with the record)

_____

1b. কোন সালে ঐ বাড়িতে প্রথম গিয়েছিল? |___|___|___|___|
1b . In what year did you move into that house (house #3, house#4 etc.)?

1c এই বাড়িতে থাকাকালীন পানির জায়গার কোন পরিবর্তন হয়েছে?

1c. Was there any change in your water source while you lived in that house (house #3, house #4 etc)? ☐ হ্যাঁ / Yes ☐ না / No → Go to Q1e

1d. আগের আগের বাড়িতে থাকাকালীন আপনাদের কতবার খাওয়া ও রান্নার জন্য পানির জায়গা বদল হয়েছিল?
1d . How many changes in drinking water sources occurred while at that house? |___|___|

1e. পানির জায়গা বদল করার আগে *খাওয়া ও রান্নার জন্য* কি একটা কল থেকে পানি আনা হত না একাধিক কল থেকে পানি আনা হত ?
1e. After the last change in water source in that *house, did you get water for drinking and cooking from just one source or more than one source (check)?*

☐ মাত্র একটি (Just one)
☐ একের বেশী (More than one) → Go to Q 2

1f. i) খাওয়া ও রান্নার জন্য পানির জায়গা কি ছিল? (What was the water source?) _____

(Probe: What was it? For example, which tubewell, pond, supply water? What was the location?)

ii) Sample collected Y / N iii) Sample code number_____

iv) Location or TW ID # .....

v) Name of the tubewell owner .....

2. খাওয়া ও রান্নার জন্য কতগুলো পানির জায়গা ছিল? (How many sources?) |___|___|

3. সব পানির জায়গা গুলো কোথায় ছিল বলুন আর কোথা থেকে কত ভাগ পানি খাওয়া ও রান্নার জন্য আনা হত তার পরিমাণ বলুন?

3. Please tell me the location of all your water sources, and how much of your drinking water you obtained from each of these sources.

(Note: Identify each source so that it can be located. Write in the location. Circle the proportion for each source and make sure the proportions add to one for all the sources combined.)

3a i) ঐ আগের বাড়িতে (H___T1) আপনাদের বেশীরভাগ সময় কোথা থেকে *খাওয়া ও রান্নার জন্য* পানি আনতেন ?

i) What was the first source of drinking and cooking water? _____

ii) ঐখান থেকে মোট কত ভাগ পানি ব্যবহার করা হত?

ii)What was the proportion of water you use from this source? <1/4, 1/4, 1/2, 3/4, >3/4

ii) Sample collected Y / N iii) Sample code number_____

iv) Location or TW ID # .....

v) Name of the tubewell owner .....

3b. i) তখন ওখানকার দ্বিতীয় পানির জায়গা (H___T1) কি ছিল?

i) What was the second source of drinking and cooking water? _____

ii) ঐখান থেকে মোট কত ভাগ পানি ব্যবহার করা হত?

ii) What was the proportion of water you use from this source? <1/4, 1/4, 1/2, 3/4, >3/4

ii) Sample collected Y / N iii) Sample code number_____

iv) Location or TW ID # .....

v) Name of the tubewell owner .....

3c. i) তখন ওখানকার আপনাদের পানির তৃতীয় জায়গা (H___T1) কি ছিল?

i) What was the third source of drinking and cooking water? _____

ii) ঐখান থেকে মোট কত ভাগ পানি ব্যবহার করা হত?

ii) What was the proportion of water you use from this source? <1/4, 1/4, 1/2, 3/4, >3/4

ii) Sample collected Y / N iii) Sample code number_____

iv) Location or TW ID # .....

v) Name of the tubewell owner .....

3d. i) তখন ওখানকার আপনাদের চর্তুথ পানির জায়গা (H___T1) কি ছিল?
i) What was the fourth source of drinking and cooking water? _____

ii) ঐখান থেকে মোট কত ভাগ পানি ব্যবহার করা হত?
ii) What was the proportion of water you use from this source? <1/4, 1/4, 1/2, 3/4, >3/4

ii) Sample collected Y / N iii) Sample code number_____

iv) Location or TW ID # .....

. v) Name of the tubewell owner .....

এই বাড়িতে থাকা অবস্থায় যদি আরো পানির উৎসের পরিবর্তনের হয় তাহলে অতিরিক্ত ১০-১১ নং পাতা সংযুক্ত করে পূরণ করুন। (যেমন- H2T2, H2T3.....)
যদি অংশগ্রহনকারী জন্মের পর থেকে আর কোন বাড়ি পরিবর্তন করে না থাকে, তাহলে ১২ নং পাতা পূরণ করুন।

If there was a CHANGE in the water source at the HOUSE before the previous one,. Insert a copy of pages 8-9 for EACH ADDITIONAL CHANGE in water source at that HOUSE, going back in time. Fill in the house and water source number at the top of the page, H3T2, H3T3, etc.

If participant has NOT LIVED IN ANY OTHER PREVIOUS HOUSES since he/she was born, go to page 12.

ENVIRONMENTAL TOBACCO SMOKE

এখন প্রশ্নগুলি হচ্ছে _____ (সন্তানের নাম)' র মা, বাবা ও অভিভাবকদের ধূমপান সর্ম্পকে।

Now, I will ask some questions about smoking habits of [child's name]'s parents or guardians.

1. _____ [সন্তানের নাম] ' র মা (বা দাদি / মহিলা অভিভাবক) কি কখনো ধূমপান করেছেন?

1. Has [child's name]'s mother (or grandmother / female guardian) ever smoked? ☐ হ্যাঁ / Yes → GO TO Q.2a
☐ না / No → GO TO Q.5

2a. _____ [সন্তানের নাম] ' র মা (বা দাদি / মহিলা গার্জিয়েন) সাধারণত কি ধরনের ধূমপান

2a. What does she usually smoke? ☐ বিড়ি /bidi ☐ সিগারেট - বাজারের /manufactured cigarettes
☐ সিগারেট - হাতে বানান /hand rolled cigarettes
☐ অন্য /other _____

2b. _____ [সন্তানের নাম] ' র মা (বা দাদি / মহিলা অভিভাবক) কি এখন বাড়ির ভেতরে সিগারেট বা বিড়ি ধূমপান করেন?

2b. Does [child's name]'s mother (or grandmother/ female guardian) currently smoke cigarettes or bidi inside the house?

☐ হ্যাঁ / Yes → GO TO Q.2c
☐ না / No → GO TO Q.3a

2c. _____ [সন্তানের নাম]' র মা (বা দাদি / মহিলা অভিভাবক) এক দিনে সাধারণত বাড়ির ভেতরে ক'টা বিড়ি বা সিগারেট ধূমপান করেন ?

2c. About how many cigarettes does [child's name]'s mother (or grandmother/ female guardian) smoke each day inside the house? |___|___| number of bidi/cigarettes

3a. _____

3a. ☐ হ্যাঁ /Yes → GO TO Q.3b

☐ না / No → GO TO Q.4a

3b. _____ 3b.

|___|___| number of bidi/cigarettes

4a. _____ [সন্তানের নাম]'র প্রথম পাঁচ বছর বয়স পর্যন্ত কি আপনি (বা দাদি / মহিলা অভিভাবক) বাড়ির ভেতরে বিড়ি বা সিগারেট ধূমপান করতেন?

4a. ☐ হ্যাঁ /Yes → GO TO Q.4b

☐ না / No → GO TO Q.5

4b. তখন আপনি _____ [সন্তানের নাম] ওর মা (বা দাদি / মহিলা অভিভাবক) এক দিনে বাড়ির ভেতরে কটা সিগারেট বা বিড়ি ধূমপান করত?

4b. |___|___|_number of bidi/cigarettes

5. _____ [সন্তানের নাম] ' র বাবা, দাদা বা পরিবারের অন্য পুরুষ কি কখনো ধূমপান করেছেন?

5. Has [child's name]'s father (or grandfather/any other male member of the family) ever smoked?

☐ হ্যাঁ /Yes → GO TO Q.6a

☐ না / No → GO TO Q.9

6a. _____ [সন্তানের নাম]' র বাবা, দাদা বা _____সাধারণত কি ধরনের ধূমপান করতেন?

6a. What does he usually smoke?

☐ বিড়ি /bidi ☐ সিগারেট - বাজারের /manufactured cigarettes

☐ সিগারেট - হাতে বানান /hand rolled cigarettes ☐ অন্য /other _____

6b. _____ [সন্তানের নাম]' র বাবা, দাদা বা _____ কি এখন বাড়ির ভেতরে সিগারেট বা বিড়ি ধূমপান করেন?

6b. Does [child's name]'s father (or grandfather/any other male member of the family) currently smoke cigarettes or bidi inside the house?

☐ হ্যাঁ /Yes → GO TO Q.6c

☐ না / No → GO TO Q.7a

6c. _____ [সন্তানের নাম]'র বাবা, দাদা বা _____ বাড়ির ভেতরে এক দিনে সাধারণত ক'টা সিগারেট বা বিড়ি ধূমপান করেন?

6c. About how many cigarettes does [child's name]'s father (or grandfather/any other male member of the family) smoke each

day inside the house?

(Circle) the number of bidi/cigarettes: 1-5 ; 6-10 ; >10 ; >20 ; >other_____

7a. _____ [সন্তানের নাম] মায়ের পেটে থাকতে ওর বাবা, দাদা বা _____ কি বাড়ির ভেতরে সিগারেট বা বিড়ি ধূমপান করত?

7a.

☐ হ্যাঁ /Yes → GO TO Q.7b

☐ না / No → GO TO Q.8a

7b. _____ [সন্তানের নাম] পেটে থাকতে ওর বাবা, দাদা বা _____ বাড়ির ভেতরে দিনে সাধারণত ক'টা সিগারেট বা বিড়ি ধূমপান করত?

7b.

(Circle) the number of bidi/cigarettes: 1-5 ; 6-10 ; >10 ; >20 ; >other_____

8a. _____ [সন্তানের নাম] 'র প্রথম পাঁচ বছর বয়স পর্যন্ত কি ওর বাবা, দাদা বা _____ বাড়ির ভেতরে সিগারেট বা বিড়ি ধূমপান করত?

8a. ☐ হ্যাঁ /Yes → GO TO Q.8b

☐ না / No → GO TO Q.9

8b. _____ [সন্তানের নাম]' র প্রথম পাঁচ বছর বয়স পর্যন্ত কি ওর বাবা, দাদা বা _____ বাড়ির ভেতরে ক'টা সিগারেট বা বিড়ি ধূমপান করত?

8b.

(Circle) the number of cigarettes/bidi: 1-5 ; 6-10 ; >10 >20 >other_____

9a. বাড়িতে সব মিলিয়ে অন্যান্য কয়জন ব্যক্তি বাড়ির ভেতরে সিগারেট বা বিড়ি ধূমপান করে?

9a. How many other people living in the house currently smoke cigarettes inside the house?|___|___| জন ব্যক্তি /people

9b. _____ [সন্তানের নাম] পেটে থাকতে পরিবারে অন্যান্য কয়জন ব্যক্তি বাড়ির ভেতরে সিগারেট বা বিড়ি ধূমপান করত?

9b.

|___|___| জন ব্যক্তি /people

9c. _____ [সন্তানের নাম] ' র প্রথম পাঁচ বছর বয়স পর্যন্ত পরিবারে অন্যান্য কয়জন ব্যক্তি বাড়ির ভেতরে সিগারেট বা বিড়ি ধূমপান করত?

9c.

|___|___| জন ব্যক্তি /people

INDOOR BIOMASS COMBUSTION

এখন কিছু প্রশ্ন করব আপনার বাড়িতে রান্না করার উনুন বা চুলা সম্পর্কে।
I would like to ask you some questions the stove you use here in this house and about method cooking.

1. বাড়িতে কি ধরনের উনুন বা চুলা ব্যবহার হয়? (What type of stove is used in the household?)

☐ তিনটে ইটের (খোলা) / Open fire (e.g. three stones) ☐ চুলা (চিমনি ছাড়া) / Traditional chulha, no chimney

☐ চুলা (চিমনি যুক্ত) / Improved chulha, with chimney ☐গ্যাস / কেরসিন / বিদ্যুৎ স্টোভ / Gas/kero/electric stove

2. উনুন বা স্টোভ কোথায় ? (Where is the stove located?)

☐ বাইরে বা আঢাকা / Outdoors **(Go to Q. 4)** ☐ অর্ধেক-বাইরে বা অর্ধেক-ঢাকা / Semi-enclosed**(Go to Q. 4)**

☐ আলাদা রান্নাঘর / Separate kitchen ☐ বসবার ঘরে / In living area

3a. রান্না যদি ঘরের ভেতর করা হয় সেখানে জানালা আছে?
3a. IF INDOORS: Is there a window in the cooking area?

☐ না কিছু নেই / None ☐ শুধু একটা / One only ☐ দুটো বা বেশী / Two or more

3b. রান্না যদি ঘরের ভেতর করা হয় সেখানে কি কোন ফুটো আছে?
3b. IF INDOORS: Is there any vent(s) in the cooking area?

☐ না কিছু নেই / None ☐ হ্যাঁ / Yes

4. আপনার রান্নার চুলাতে প্রধানত কি ধরনের জ্বালানী ব্যবহার হয়? / What type of fuel is used with the stove?

☐ কাঠ / Firewood ☐ শোকানো পাতা শস্য যেমন বিচালি বা কাঠের গুড়ো / Crop residue (like straw) or wood chips

☐ গইডা / Dungcakes ☐ কাঠকয়লা / Charcoal ☐ গ্যাস / LPG

☐ কয়লা / Coal ☐ কেরসিন / Kerosene ☐ বিদ্যুৎ / Electricity

☐ পাটখড়ি / সোলা

5. _____ [সন্তানের নাম] দিনে সাধারণ কত সময় রান্নাঘরে কাটায় ?
5. How much time does [child's name] spend in the kitchen during a normal day? |___|___|.|___|___|

ঘন্টা / hours

6. সাধারণত দিনে কতবার রান্না করা হয় (সকালের নাস্তা সহ)?

6. How many meals, including breakfast, are cooked per day in your family? |___|___| খাবার / meals

7. বর্তমান জ্বালানীর আগে কি ধরনের জ্বালানী ব্যবহার হত?
7. What fuel did you use before the current type of fuel? _____

এখন কিছু প্রশ্ন করব কি ধরনের উনুন বা চুলা ব্যবহার হত যখন _____ [সন্তানের নাম] জন্মেছিল ।
Now I will ask you some questions about the stove you used when [child's name] was born.

8. যখন _____ [সন্তানের নাম] জন্মেছিল তখন কি ধরনের উনুন বা চুলা ব্যবহার হত?
8. What type of stove was used in the household when [child's name] was born?

☐ তিনটে ইটের (খোলা) / Open fire (e.g. three stones) ☐ চুলা (চিমনি ছাড়া) / Traditional chulha, no chimney

☐ চুলা (চিমনি যুক্ত) / Improved chulha, with chimney ☐ গ্যাস / কেরসিন / বিদ্যুৎ স্টোভ / Gas/kero/electric stove

9. যখন _____ [সন্তানের নাম] জন্মেছিল তখন উনুন বা স্টোভ কোথায় ছিল ?
9. Where was the stove located when [child's name] was born?

☐ বাইরে বা আঢাকা / Outdoors**(Go to Q. 11)** ☐ অর্ধেক-বাইরে বা অর্ধেক-ঢাকা / Semi-enclosed**(Go to Q. 11)**

☐ আলাদা রান্নাঘর / Separate kitchen ☐ বসবার ঘরে / In living area

10. রান্না যদি ঘরের ভেতর করা হয় সেখানে জানালা বা কোন ফুটো আছে?
10. IF INDOORS: Is there a window or vent in the cooking area?

☐ না কিছু নেই / None ☐ শূধু একটা / One only ☐ দুটোর বা বেশী / Two or more

11. তখন রান্না উনুন বা চুলাতে কি ধরনের জ্বালানী ব্যবহার হত? / What type of fuel was used with the stove?

☐ কাঠ / Firewood ☐ শোকানো পাতা শস্য যেমন বিচালি বা কাঠের গুড়ো / Crop residue (like straw) or wood chips

☐ গইডা / Dungcakes ☐ কাঠকয়লা / Charcoal ☐ গ্যাস / LPG ☐ পাটখড়ি / সোলা

☐ কয়লা / Coal ☐ কেরসিন / Kerosene ☐ বিদ্যুৎ / Electricity

12. _____ [সন্তানের নাম]' র প্রথম এক বছর বয়স পর্যন্ত সাধারণ দিনে কত সময় রান্না ঘরে (মায়ের সাথে) কাটাত?
12. How much time did [child's name] spend in the kitchen (with her mother) during a normal day when he/she was an infant (less than 1 year old)?

|___|___|.|___|___| ঘন্টা / hours

13. _____ [সন্তানের নাম]' র পেটে থাকতে ওর মা দিনে রান্না ঘরে কত সময় কাটাত?
13. How much time did (child's name) mother spend in the kitchen during a normal day when she was pregnant with [child's name]?

|___|___|.|___|___| ঘন্টা / hours

SOCIO-DEMOGRAPHIC CHARACTERISTICS

এখন কিছু প্রশ্ন করব আপনার লেখা পড়া, বাড়ির কিছু জিনিস পত্র সম্পর্কে।
I will now ask you questions about your level of education, some of the items you might have in your house and some features of your house.

1. আপনি কতদূর লেখা পড়া করেছেন ?
1. What is the highest level of education you have attained?

☐ No education
☐ Primary (KG to class 5) _____
☐ Secondary (6 to 10) _____
☐ Madrasa _____

☐ Higher Secondary
☐ Vocational/Technical Institute
☐ Polytechnic
☐ Bachelor Degree
☐ Masters Degree
☐ Other _____

2. আপনার ঘরের প্রধান ব্যক্তি সে সর্বোচ্চ কোন স্তর অবধি পড়েছেন ?
2. What is the highest level of education attained by the head of your household?

☐ No education
☐ Primary (KG to class 5) _____
☐ Secondary (6 to 10) _____
☐ Madrasa _____

☐ Higher Secondary
☐ Vocational/Technical Institute
☐ Polytechnic
☐ Bachelor Degree
☐ Masters Degree
☐ Other _____

3. আপনার সন্তান যে ঘরে বাস করে তা কিসের তৈরী?
3. What is the building material of the house the child lives in?

☐ concrete/brick (Pucca) ☐ mud/tin/thatched hut (Kacha)

☐ combination of high quality and low quality materials (Semi-pucca) ☐ tin(concrete floor)

4. আপনার বাড়িতে কটা রুম আছে, রান্না ঘর, পায়খানা, গুদাম ছাড়া ?
4. How many rooms are there in the house the child lives in (not including kitchen, toilets, storage)?

|___|___| rooms

5. _____ [সন্তানের নাম] যে বাড়িতে জন্মেছিল সেই বাড়িটি কেমন ছিল ?
5. What was the building material of the house the child was born in?

☐ concrete/brick (Pucca) ☐ mud/thatched hut/ tin (Kacha)
☐ combination of high quality and low quality materials (Semi-pucca) ☐ tin (concrete floor)

6. _____ [সন্তানের নাম] যে বাড়িতে জন্মেছিল সেই বাড়িতে কটা রুম ছিল, রান্না ঘর, পায়খানা, গুদাম ছাড়া ?
6. How many rooms were there in the house the child was born in (not including kitchen, toilets, storage)?

|___|___| rooms

Ending time: |___|___| . |___|___| AM/PM

Name of the interviewer: _____

Signature of the interviewer: _____

Appendix-3b: Parental main questionnaire (Pdf)

Arsenic exposure in utero and early life and health effects in children and Adolescent Study ID |__|__|__|

PARENTAL MAIN QUESTIONNAIRE

CID: |__|__|__|__|__|__|__|__|__|__|

RID: |__|__|__|__|__|__|__|__|__|__|

Starting Time: |__|__|.|__|__| AM/PM **Date:** |__|__|__|__|__|__| DD/MM/YY

Respondent will be the primary caretaker (usually the mother) of the participant. For questions with [child's name], please insert the participant's name when asking the question.

Respondent's relationship to participant:_____

এখন আমি কিছু সাধারন প্রশ্ন জিজ্ঞেস করব---------- (সন্তানের নাম)'র আর তাদের পরিবার সম্পর্কে
I am going to start by asking you some general questions about [child's name] and your family.

1. _____ (সন্তানের নাম) বয়স কত? / What is [child's name]'s age? |__|__| Years

(If the parent is not sure about participant's birth date, ask the following questions.)

2. _____ (সন্তানের নাম) কোন ক্লাসে পড়ে? |__|__|
2. Which grade school is [child's name] in currently?
"NA" means the child does not goes to school currently.

3. বড়, ছোট মিলিয়ে আপনার পরিবারে মোট কতজন একই ঘরে বাস করেন? |__|__|
3. How many family members live in your current house (include both children and adults)?

RESIDENCE HISTORY

(Keep inserting the years, house and water source numbers as you ask the following questions in the time log boxes on the loose sheet. Staple the sheet at the end of the interview. This is to work out the historical drinking and cooking water exposure throughout the participant's life time. The detailed questions are on the following pages.)

1. _____ (সন্তানের নাম) কোন সালে জন্মেছিল?

(Insert the year on the line above, at the spot noted by YOB of the child)

2. (সন্তানের নাম) জন্মাবার পর থেকে কি এই বাড়িতেই আছেন?
2. Have you been staying in the same house since our last visit? ☐ Yes → Go to page 2.
☐ / No

3. _____ (সন্তানের নাম) জন্মাবার পর কতগুলি বাড়িতে থেকেছেন? (৬ মাস অথবা অধিক) |__|__|
3. How many houses have you lived in since our last visit(6 months or more)?

RESIDENTIAL WATER HISTORY - CURRENT HOUSE, CURRENT SOURCE
SECTION H1T1

Appendix 4a: Child Main questionnaire

School/Madrasa Water History

CID: |___|___|___|___|___|___|___|___|___|

RID: |___|___|___|___|___|___|___|___|___|___|

Starting Time: |___|___|.|___|___| AM/PM **Date:** |__|__|__|__|__|__| DD/MM/YY

SCHOOL/MADRASA HISTORY

এখন আমি তোমার স্কুল/ মাদ্রাসার সম্পর্কে কিছু প্রশ্ন করব।
Now, we would like to ask you about your school/madrasa.

১. তুমি কি বর্তমানে স্কুলে/ মাদ্রাসায় যাও?
1. Do you go to school (currently)?

☐ হ্যা, বর্তমানে স্কুলে / মাদ্রাসায় যায় **Go to Q 3.**
☐ না, বর্তমানে স্কুলে / মাদ্রাসায় যায় না **Go to Q 2.**
☐ কখনো স্কুলে / মাদ্রাসায় যায়নি **Go to Page 11.**

2. কোন ক্লাস পর্যন্ত পড়েছিলে? |___|___|
2. In which class did you read?

3. তুমি কখনো বাড়ির বাইরে থেকে কমপক্ষে ছয় মাস স্কুলে / মাদ্রাসায় গেছ?
3. Have you gone to school/madrasa outside your house for at least 6 months?
☐ হ্যা /Yes ☐ না / No

4. তুমি কোন কোন স্কুলে / মাদ্রাসায় পড়েছ, বর্তমান থেকে শুরু করে একেবারে প্রথম যে স্কুলে / মাদ্রাসায় পড়েছ তার নাম বলো
4. Please tell me what school/madrasas you attended, starting from the current school/madrasa and going back in time.

Note: List the school/madrasas in the table on the next page. Only identify major changes of location. Start with the most recent, and move back in time to the earliest. Use as many lines as needed.

Note: After filling out the table, complete School/Madrasa Water Source History section for each school/madrasa and each water source, starting with the most recent. Numbering of the following pages is as follows:

Current school/madrasa, current water source:	**Section S1T1**
Current school/madrasa, second to last water source:	**Section S1T2**
Current school/madrasa, third to last water source:	**Section S1T3, etc.**
Prior to current school/madrasa, final water source:	**Section S2T1**
Prior to current school/madrasa, second to last water source:	**Section S2T2, etc.**

School/Madrasa Water History

স্কুলের কার্যক্ষেত্র (বর্তমান থেকে শুর) School/madrasa Activity **Note: Start with most recent school/madrasa.**	কোন বৎসরে এই স্কুলে তোমার যাওয়া শুর হয়েছিল? In what year did you start this school/madrasa?	কোন বৎসরে এই স্কুলে তোমায় যাওয়া শেষ হয়েছিল? In what year did you stop attending this school/madrasa?	শুর করার সময় তোমার কত বয়স ছিল? What was your age when you started attending?	শেষ করার সময় তোমার কত বয়স ছিল What was your age when you stopped attending?
তোমার বর্তমান স্কুলের নাম কি? (What is your current school/madrasa?)				
S1) স্কুল/মাদ্রসা র নাম (Name of School/madrasa1) _____	|___|___|___|___|	|___|___|___|___|	|___|___|	|___|___|
এর আগে কোন স্কুলে যেতে? (What school/madrasa did you attend before that?)				
S2) স্কুল/মাদ্রসা র নাম (Name of School/madrasa 2) _____	|___|___|___|___|	|___|___|___|___|	|___|___|	|___|___|
ঐ স্কুলের আগে কোন স্কুলে যেতে? (What school/madrasa did you attend before _____(fill in with School/madrasa #2)?				
S3) স্কুল/মাদ্রসা র নাম (Name of School/madrasa 3) _____	|___|___|___|___|	|___|___|___|___|	|___|___|	|___|___|
তার আগে কোন স্কুলে যেতে? (What school/madrasa did you attend before _____(fill in with School/madrasa #3)?				
S4) স্কুল/মাদ্রসা র নাম (Name of School/madrasa 3) _____	|___|___|___|___|	|___|___|___|___|	|___|___|	|___|___|

SCHOOL/MADRASA WATER SOURCE HISTORY--CURRENT SCHOOL/MADRASA
CURRENT SOURCE **S1T1**

1. স্কুলের বা মাদ্রাসার নং (School/madrasa number from Page 2): _____

2. তুমি স্কুল বা মাদ্রাসায় যে পানি খাও সব কি বাড়ি থেকে নিয়ে যাও?
2. Do you take all your water from home to your current school/madrasa?

☐ হ্যা/Yes → Go to Page 7.

☐ না / No →Go to Q 3

☐ স্কুলে পানি খায় না / Does not take water at school → Go to Page 7

3. স্কুলের বা মাদ্রাসার ঠিকানা কি?
3. What is the address of the school/madrasa?

বাড়ির নাম (Name of the house) _____

(Nearest landmark) _____

গ্রাম (Village) _____

4a. স্কুলে তুমি কি একটি মাত্র উৎস থেকে পানি খাও নাকি একাধিক উৎস থেকে পানি খাও?

4a. In school/madrasa, do you get drinking water from just one source or more than one source (check)?

☐ মাত্র একটি (Just one) ☐ একের বেশী (More than one) → Go to Q 4c

4b. i) পানির উৎস কি? কোথা থেকে পানি খাও? (কল না পাত কুয়ো না পুকুর না টাইম কল?)

4b. i) What is the water source? _____

(Probe: What is it? For example, which tubewell, pond? What is the location?)

ii) Sample collected Y / N iii) Sample code number_____

iv) Location or TW ID # ……

or Name of the tube well owner ……

4c. কতগুলো উৎস থেকে পানি খাও? |___|

4c. (How many sources?)

5. সব পানির উৎস গুলো কোথায় বলো আর কোথায় থেকে কত ভাগ পানি খাও, তার পরিমাণ বলো?

5. Please tell me the location of all your water sources, and how much of your drinking water you obtain from each of these sources.

Note: Identify each source so that it can be located. Write in the location. Circle the proportion for each source and make sure the proportions add to one for all the sources combined.

5a. i) তুমি সবচেয়ে বেশী পানি কোন উৎস থেকে খাও?

i) What is the primary source of drinking water? _____

ii) এই পানির উৎস থেকে কত পরিমাণ পানি খাওয়া হয়?

ii) What is the proportion of water you use from this source? <1/4, 1/4, 1/2, 3/4, >3/4

iii) Sample collected Y / N iv) Sample code number_____

v) Location……

or Name of the tubewell owner ……

5b. i) তুমি দ্বিতীয়ত কোন উৎস থেকে পানি খাও?

5b. i) What is the second source of drinking water? _____

ii) ঐ উৎস থেকে কি পরিমাণ পানি খাওয়া হয়?

ii) What is the proportion of water you use from this source? <1/4, 1/4, 1/2, 3/4, >3/4

iii) Sample collected Y / N iv) Sample code number_____

v) Location……

or Name of the tubewell owner ……

5c. i) তোমার তৃতীয় পানির উৎস কি?

ii) What is the third source of drinking water?)_____

ii) এই পানির উৎস থেকে কত পরিমাণ পানি খাওয়া হয়?

ii) What is the proportion of water you use from this source? <1/4, 1/4, 1/2, 3/4, >3/4

iii) Sample collected Y / N iv) Sample code number_____

v) Location……

……

5d. i) তোমার চর্তুথ পানির উৎস কি?
ii) What is the fourth source of drinking water?)_____

ii) এই পানির উৎস থেকে কত পরিমাণ পানি খাওয়া হয়?
ii) What is the proportion of water you use from this source? <1/4, 1/4, 1/2, 3/4, >3/4

iii) Sample collected Y / N iv) Sample code number_____

v) Location……

……

পরবর্তি প্রশ্নের উত্তরে 'হ্যা' কিংবা 'না' উত্তর দাও।
Please answer the next question with a "yes" or "no".

6. এই বর্তমান স্কুলে পড়াকালীন তোমার খাবার পানির উৎসর কি কোন বদল হয়েছিল?
Was there any change in your drinking water source while you attended your current school/madrasa?

☐ হ্যাঁ / Yes **Fill out pages 5-6 IF THERE WAS A CHANGE in the water source at the CURRENT SCHOOL/MADRASA while the participant attended current school/madrasa. Insert a copy of pages 5-6 for EACH ADDITIONAL CHANGE in water source at the CURRENT SCHOOL/MADRASA, going back in time. Fill in the water source number on the upper right corner, S1T2, S1T3 etc.**

☐ না / No **If the participant has ONLY ONE SCHOOL/MADRASA listed on page 2, go to page 11.**

If participant has MORE THAN ONE SCHOOL/MADRASA listed on page 2, fill out pages 7-8. Insert a copy of pages 7-8 for EACH ADDITIONAL SCHOOL/MADRASA listed, going back in time. Fill in the school/madrasa number on the upper right corner, S2T1, S3T1 etc.

SCHOOL/MADRASA WATER HISTORY – CURRENT SCHOOL/MADRASA, CHANGE IN WATER SOURCE	**S1T ☐**	**(Fill in source number)**

Note: Insert a copy of pages 5-6 for EACH CHANGE in water source at the CURRENT SCHOOL/MADRASA, going back in time. Fill in source number on the top of the page.

1a. এই বর্তমান স্কুলে থাকাকালীন তোমার পানির উৎস কত বার বদল হয়েছে?
1a. How many changes in drinking water sources occurred while at your current school/madrasa?
_____

1b. কোন বৎসর তুমি এই পরবর্তি পানির উৎস বদল করেছ?
1b. In what year did the change in drinking water source occur? _____

1c. তখন তোমার বয়স কত ছিল?
1c. How old were you then? _____ Years

Note: If there is a discrepancy in Q1, please clarify it with the participant.

2a. স্কুলে পানির উৎস বদল হবার আগে, তুমি কি একটি মাত্র উৎস থেকে পানিখেতে না একাধিক উৎস থেকে পানিখেতে?
2a. Before the change in water source, did you get drinking water from just one source or more than one source (check)? ☐ মাত্র একটি (Just one) → Go to Q.2b
☐ একের বেশী (More than one) → Go to Q.2c

2b. i) পানির উৎস কি ছিল? কোথা থেকে পানিখেতে? (কল না পাত কুয়ো না পুকুর, না টাইম কল?)
2b. i) What was the water source?
(Probe: What is it? For example, which tubewell, pond? What is the location?) _____

ii) Sample collected Y / N iii) Sample code number_____

iv) Location or TW ID # ……

or Name of the tubewell owner……

2c. কতগুলো উৎস থেকে পানিখেতে? (How many sources?) _____

3. সব পানির উৎস গুলো কোথায় বলো আর কোথার থেকে কত ভাগ পানিখেতে তার পরিমাণ বলো?
3. Please tell me the location of all your water sources, and how much of your drinking water you obtained from each of these sources.

Note: Identify each source so that it can be located. Write in the location. Circle the proportion for each source and make sure the proportions add to one for all the sources combined.

3a. i) তোমার প্রথম পানির উৎস কি?
i) What was the first source of drinking water?_____

ii) এই পানির উৎস থেকে কত পরিমাণ পানিখেতে?
ii) What was the proportion of water you use from this source? <1/4, 1/4, 1/2, 3/4, >3/4

iii) Sample collected Y / N iv) Sample code number_____

iv) Location or TW ID # ……

or Name of the tubewell owner……

3b. i) তোমার দ্বিতীয় পানির উৎস কি?
i) What was the second source of drinking water? _____

ii) এই পানির উৎস থেকে কত পরিমাণ পানিখেতে হয়?
ii) What was the proportion of water you use from this source? <1/4, 1/4, 1/2, 3/4, >3/4

iii) Sample collected Y / N iv) Sample code number_____

iv) Location or TW ID # ……

or Name of the tubewell owner……

3c. i) তোমার তৃতীয় পানির উৎস কি?
i) What was the third source of drinking water?_____

ii) এই পানির উৎস থেকে কত পরিমাণ পানি খাওয়া হয়?
ii) What was the proportion of water you use from this source? <1/4, 1/4, 1/2, 3/4, >3/4

iii) Sample collected Y / N iv) Sample code number_____

iv) Location or TW ID # ……

or Name of the tubewell owner……

3d. i) তোমার চর্তুথ পানির উৎস কি?
i) What was the fourth source of drinking water? _____
ii) এই পানির উৎস থেকে কত পরিমাণ পানি খাওয়া হয়?
ii) What was the proportion of water you use from this source? <1/4, 1/4, 1/2, 3/4, >3/4

iii) Sample collected Y / N iv) Sample code number_____

iv) Location or TW ID # ……

or Name of the tubewell owner……

4. পরবর্তি প্রশ্নের উত্তরে 'হ্যা' কিংবা 'না' উত্তর দাও । এই বর্ত্তমান স্কুলে তোমার খাবার পানির উৎসর কি আর কোন বদল হয়েছিল?
4. Was there any other change in your drinking water source while you attended your current school/madrasa?

☐ হ্যাঁ / Yes **Insert a copy of pages 5-6 for EACH ADDITIONAL CHANGE in water source at the CURRENT SCHOOL/MADRASA, going back in time. Fill in the water source number on the upper right corner, S1T2, S1T3 etc.**

☐ না / No **If the participant has ONLY ONE SCHOOL/MADRASA listed on page 2, go to page 11.**

If participant has MORE THAN ONE SCHOOL/MADRASA listed on page 2, fill out pages 7-8. Insert a copy of pages 7-8 for EACH ADDITIONAL SCHOOL/MADRASA listed, going back in time. Fill in the school/madrasa number on the upper right corner, S2T1, S3T1 etc.

SCHOOL/MADRASA WATER SOURCE HISTORY – PREVIOUS SCHOOL/MADRASAS, LAST SOURCE **Section S☐T1** **(Fill in school/madrasa number)**

Note: Insert a copy of pages 7-8 for EACH SCHOOL/MADRASA listed, going back in time. Fill in the school/madrasa number on the top of the page.

1. স্কুলের নং (School/madrasa number from Page 2): _____

2. তুমি আগের স্কুলে যা পানিখেতে সব কি বাড়ির থেকে নিয়ে যেতে?
2. Did you take all your water from home to your previous school/madrasa?)

☐ হ্যাঁ /Yes → Go to Q 6. (instructions)

☐ না / No →Go to Q.3

☐ স্কুলে পানি খায় নি/ Did not take water at school → Go to Q 6. . (instructions)

স্কুলের ঠিকানা কি ছিল?

3. What is the address of the school/madrasa? পারা (Para) _____

পোস্ট অফিস (Post office) _____

গ্রাম (Village) _____

4a. ঐ স্কুলে তুমি কি একটি মাত্র উৎস থেকে পানিখেতে না একাধিক উৎস থেকে পানিখেতে?

4a. In that school/madrasa, did you get drinking water from just one source or more than one source (check)?

☐ মাত্র একটি (Just one) → Go to Q.4b

☐ একের বেশী (More than one) → Go to Q.4c

4b. i) পানির উৎস কি ছিল? কোথা থেকে পানিখেতে? (কল না পাত কুয়ো না পুকুর, না টাইম কল?)

4b. i) What was the water source? _____

(Probe: What is it? For example, which tubewell, pond? What is the location?)

ii) Sample collected Y / N iii) Sample code number_____

iv) Location or TW ID # ……

or Name of the tubewell owner ……

……

4c. কতগুলো উৎস থেকে পানিখেতে?

4c. How many sources? _____

5. সব পানির উৎস গুলো কোথায় ছিল বলো আর কোথার থেকে কত ভাগ পানিখেতে তার পরিমাণ বলো?

5. Please tell me the location of all your water sources, and how much of your drinking water you obtained from each of these sources.

Note: Identify each source so that it can be located. Write in the location. Circle the proportion for each source and make sure the proportions add to one for all the sources combined.

5a. i) তোমার প্রথম পানির উৎস কি?

i) What was the first source of drinking water? _____

ii) এই পানির উৎস থেকে কত পরিমাণ পানি খাওয়া হত?

ii) What was the proportion of water you use from this source? <1/4, 1/4, 1/2, 3/4, >3/4

iii) Sample collected Y / N iv) Sample code number_____

v) Location……

Name of the tubewell owner……

5b. i) তোমার দ্বিতীয় পানির উৎস কি?

i) What was the second source of drinking water? _____

ii) এই পানির উৎস থেকে কত পরিমাণ পানি খাওয়া হত?

ii) What was the proportion of water you use from this source? <1/4, 1/4, 1/2, 3/4, >3/4

iii) Sample collected Y / N iv) Sample code number_____

iv) Location or TW ID # ……

or Name of the tubewell owner……

5c. i) তোমার তৃতীয় পানির উৎস কি?
i) What was the third source of drinking water? _____

ii) এই পানির উৎস থেকে কত পরিমাণ পানি খাওয়া হত?
ii) What was the proportion of water you use from this source? <1/4, 1/4, 1/2, 3/4, >3/4

iii) Sample collected Y / N iv) Sample code number_____

iv) Location or TW ID # ……

Name of the tubewell owner……

5d. i) তোমার চর্তুথ পানির উৎস কি?
i) What was the fourth source of drinking water? _____

ii) এই পানির উৎস থেকে কত পরিমাণ পানি খাওয়া হয়?
ii) What was the proportion of water you use from this source? <1/4, 1/4, 1/2, 3/4, >3/4

iii) Sample collected Y / N iv) Sample code number_____

iv) Location or TW ID # ……

or Name of the tubewell owner……

পরবর্তি প্রশ্নের উত্তরে 'হ্যা' কিংবা 'না' উত্তর দাও ।
Please answer the next question with a "yes" or "no".

6. যখন তুমি _____ স্কুলে যেতে তখন তোমার খাবার পানির উৎসর কি আর বদল হয়েছিল?
Was there any change in your drinking water source while you attended? _____(fill in the appropriate school/madrasa number)?

☐ হ্যাঁ / Yes **Fill out pages 9-10 IF THERE WAS A CHANGE in the water source at the PREVIOUS SCHOOL/MADRASA while the participant attended that school/madrasa. Insert a copy of pages 9-10 for EACH ADDITIONAL CHANGE in water source at the PREVIOUS SCHOOL/MADRASA, going back in time. Fill in the school/madrasa and water source number on the upper right corner, S2T2, S2T3 etc.**

☐ না / No **If the participant does not have any more school/madrasas listed on page 2, go to page 11.**

Insert a copy of pages 7-8 for EACH ADDITIONAL SCHOOL/MADRASA listed, going back in time. Fill in the school/madrasa number on the upper right corner, S2T1, S3T1 etc.

SCHOOL/MADRASA WATER HISTORY – PREVIOUS SCHOOL/MADRASAS, CHANGE IN WATER SOURCE SECTION S☐T☐ (Fill in school/madrasa source number)

Note: Insert a copy of pages 9-10 for EACH CHANGE in water source at the PREVIOUS SCHOOL/MADRASA, going back in time. Fill in school/madrasa number and source number on the top of the page.

1a. _____ স্কুলে থাকাকালীন তোমার পানির উৎস কত বার বদল হয়েছে? _____
1a. How many changes in drinking water sources occurred while at school/madrasa #_____ (fill in appropriate school/madrasa number)?

1b. কোন বৎসর তুমি পরবর্তি পানির উৎস বদল করেছ?
1b. In what year did the change in drinking water source occur? _____

1c. তখন তোমার বয়স কত ছিল? How old were you then? _____ Years

Note: If there is a discrepancy in Q1, please clarify it with the participant.

2a. ঐ স্কুলে তুমি কি একটি মাত্র উৎস থেকে পানিখেতে না একাধিক উৎস থেকে পানিখেতে?
2a. In that school/madrasa, did you get drinking water from just one source or more than one source (check)?

☐ মাত্র একটি (Just one) → Go to Q2b

☐ একের বেশী (More than one) → Go to Q2c

2b. i) পানির উৎস কি ছিল? কোথা থেকে পানিখেতে? (কল না পাত কুয়ো না পুকুর, না টাইম কল?)
2b. i) What was the water source? _____

(Probe: What is it? For example, which tubewell, pond? What is the location?)

ii) Sample collected Y / N iii) Sample code number_____
iv) Location or TW ID # .....

or Name of the tubewell owner.....

2c. কতগুলো উৎস থেকে পানিখেতে? (How many sources?) _____

3. সব পানির উৎস গুলো কোথায় ছিল বলো আর কোথার থেকে কত ভাগ পানিখেতে তার পরিমাণ বলো?
3. Please tell me the location of all your water sources, and how much of your drinking water you obtained from each of these sources.

Note: Identify each source so that it can be located. Write in the location. Circle the proportion for each source and make sure the proportions add to one for all the sources combined.

3a. i) তোমার প্রথম পানির উৎস কি ছিল ?
i) What was the first source of drinking water? _____

ii) এই পানির উৎস থেকে কত পরিমাণ পানি খেতে?
ii) What was the proportion of water you use from this source? <1/4, 1/4, 1/2, 3/4, >3/4

iii) Sample collected Y / N iv) Sample code number_____
iv) Location or TW ID # .....

or Name of the tubewell owner.....

3b. i) তোমার দ্বিতীয় পানির উৎস কি ছিল?
i) What was the second source of drinking water? _____

ii) এই পানির উৎস থেকে কত পরিমাণ পানি খতে?
ii) What was the proportion of water you use from this source? <1/4, 1/4, 1/2, 3/4, >3/4

iii) Sample collected Y / N iv) Sample code number_____
iv) Location or TW ID # .....

or Name of the tubewell owner.....

3c. i) তোমার তৃতীয় পানির উৎস কি ছিল ?

i) What was the third source of drinking water? _____

ii) এই পানির উৎস থেকে কত পরিমাণ পানি খেতে?

ii) What was the proportion of water you use from this source? <1/4, 1/4, 1/2, 3/4, >3/4

iii) Sample collected Y / N iv) Sample code number_____

iv) Location or TW ID # .....

or Name of the tubewell owner.....

3d. i) তোমার চর্তুথ পানির উৎস কি ছিল ?

i) What was the fourth source of drinking water? _____

ii) এই পানির উৎস থেকে কত পরিমাণ পানি পানি খেতে??

ii) What was the proportion of water you use from this source? <1/4, 1/4, 1/2, 3/4, >3/4

iii) Sample collected Y / N iv) Sample code number_____

iv) Location or TW ID # .....

or Name of the tubewell owner.....

4. পরবর্তি প্রশ্নের উত্তরে 'হ্যা' কিংবা 'না' উত্তর দাও । যখন তুমি _____ স্কুলে যেতে তখন তোমার খাবার পানির উৎসর কি আর বদল হয়েছিল?

4. Was there any other change in your drinking water source while you attended _____ (fill in the appropriate school/madrasa number)?

☐ হ্যাঁ / Yes **Insert a copy of pages 9-10 for EACH ADDITIONAL CHANGE in water source at the PREVIOUS SCHOOL/MADRASA, going back in time. Fill in the school/madrasa number and water source number on the upper right corner, S2T2, S2T3 etc.**

☐ না / No **If the participant does not have any more school/madrasas listed on page 2, go to page 11.**

Insert a copy of pages 7-8 for EACH ADDITIONAL SCHOOL/MADRASA listed, going back in time. Fill in the school/madrasa number on the upper right corner, S2T1, S3T1 etc.

ACTIVE SMOKING

এখন প্রশ্নগুলি হচ্ছে তোমার ধূমপান সর্ম্পকে।

Now, I will ask some questions about your smoking habits.

3a. তুমি কি এখন সিগারেট বা বিড়ি খাও? ☐ হ্যা /Yes

3a. Do you smoke cigarettes or bidi? ☐ না / No Skip Q 3b and 3c

3b. তুমি সাধারনত কি ধূমপান কর?

3b. What do you usually smoke?

☐ বিড়ি (bidi)

☐ সিগারেট (manufactured cigarettes)

3c. তুমি দিনে কয়টা সিগারেট খাও?

3c. About how many cigarettes do you smoke each day? |___|___|

Ending time: |___|___| . |___|___| AM/PM

Signature of the interviewer: _____

Interviewer Code: |___|___|

Appendix-4b: Child main questionnaire

Arsenic in utero and early life and health effects in children and adolescent Study ID |__|__|__|

School/Madrasa Water History

CID: |__|__|__|__|__|__|__|__|__|__|

RID: |__|__|__|__|__|__|__|__|__|__|

Starting Time: |__|__| : |__|__| AM/PM **Date:** |__|__|__|__|__|__| DD/MM/YY

SCHOOL/MADRASA HISTORY

এখন আমি তোমার স্কুল/ মাদ্রাসার সম্পর্কে কিছু প্রশ্ন করব
Now, we would like to ask you about your school/madrasa.

১. তুমি কি বর্তমানে স্কুলে/ মাদ্রাসায় যাও?
1. Do you go to school (currently)?

☐ হ্যাঁ, বর্তমানে স্কুলে / মাদ্রাসায় যায় **Go to Q 3.**
☐ না, বর্তমানে স্কুলে / মাদ্রাসায় যায় না **Go to Q 2.**
☐ কখনো স্কুলে / মাদ্রাসায় যাইনি **Go to Page 11.**

2. কোন ক্লাস পর্যন্ত পড়েছিলে? |__|__|
2. In which class did you read?

3. তুমি কখনো বাড়ির বাইরে গিয়ে অন্তত ছয় মাস স্কুলে / মাদ্রাসায় গেছ?
3. Have you gone to school/madrasa outside your house for at least 6 months?

☐ হ্যাঁ/Yes ☐ না/No

4. তুমি কোন কোন স্কুল / মাদ্রাসায় পড়েছ, বর্তমান থেকে শুরু করে একেবারে প্রথম যে স্কুল / মাদ্রাসায় পড়েছ তার নাম বলো
4. Please tell me what school/madrasas you attended, starting from the current school/madrasa and going back in time.

Note: List the school/madrasas in the table on the next page. Only identify major changes of location. Start with the most recent, and move back in time to the earliest. Use as many lines as needed.

Note: After filling out the table, complete School/Madrasa Water Source History section for each school/madrasa and each water source, starting with the most recent. Numbering of the following pages is as follows:

Current school/madrasa, current water source:	**Section S1T1**
Current school/madrasa, second to last water source:	**Section S1T2**
Current school/madrasa, third to last water source:	**Section S1T3, etc.**
Prior to current school/madrasa, final water source:	**Section S2T1**
Prior to current school/madrasa, second to last water source:	**Section S2T2, etc.**

1

Appendix-5a: Respiratory Health Questionnaire

RESPIRATORY HEALTH QUESTIONNAIRE

Starting Time: |___|___|. |___|___| AM/PM **Date:** |__|__|__|__|__|__| DD/MM/YY

Respondent's relationship to participant:_____ CID: |___|___|___|___|___|___|___|___|___|

RID: |___|___|___|___|___|___|___|___|___|___|

1. এখন আমি কিছু প্রশ্ন জিজ্ঞাসা করব _____(সন্ড়নের নাম)'র কখনো কাশি হয়েছে কি না সে সম্পর্কে ।
1. Now I would like to ask a few questions about any coughing [child's name] might have experienced.

1a. গত বারো মাসে, _____ (সন্ড়নের নাম) 'র কি সর্দির কারনে বুকে কফ জমা বা কাশির সঙ্গে কফ বের হয়েছিল?
1a. In the last 12 months, has [child's name] usually seemed congested in the chest or coughed up phlegm (mucus) when he/she had a cold?

☐ হ্যাঁ /Yes
☐ না / No

1b. গত বারো মাসে _____ (সন্ড়নের নাম) কি সর্দি ছাড়া বুকে কফ জমা বা কাশির সঙ্গে কফ বের হয়েছে ?
1b. In the last 12 months, has [child's name] usually seemed congested in the chest or coughed up phlegm (mucus) when he/she did not have a cold?

☐ হ্যাঁ / Yes
☐ না / No → GO TO Q.2

1c. গত বারো মাসে , _____ (সন্ড়নের নাম) কি সপ্তাহের বেশীরভাগ দিন (সপ্তাহে ৪ বা অধিক দিন) একনাগারে ৩ মাস, বুকে কফ জমা বা কাশির সঙ্গে কফ বের হয়েছে ?
1c. In the last 12 months, has [child's name] usually seemed congested in the chest or coughed up phlegm (mucus) on most days (4 or more days a week) for as much as 3 months of the year?

☐ হ্যাঁ / Yes
☐ না / No → GO TO Q.2

1d. কত বছর এ রকম হয়েছে ? For how many years has this happened? |___|___| বছর (Years)

2. গত বারো মাসে , _____ (সন্ড়নের নাম) রাত্রে বেলায় কি সর্দি ছাড়া শুকনো কাশি হয়েছে ?
2. In the last 12 months, has [child's name] had a dry cough at night, apart from a cough associated with a cold or chest infection?

☐ হ্যাঁ /Yes

☐ না / No

Wheezing

3. এখন কিছু প্রশ্ন জিজ্ঞাসা করব _____ (সন্ড়নের নাম) বুকে কোন সাঁই সাঁই/ পন পন শব্দ বা একটুতে হাঁপিয়ে যাওয়ার অভিজ্ঞতার সম্পর্কে।
3. I would like to ask some questions about any wheezing or breathlessness [child's name] may have ever experienced.

3a. _____ (সন্ড়নের নাম) কি **অতীতে / জীবনে কখনও** নি:শ্বাস নেবার সময় বুকের মধ্যে সাঁই সাঁই/ পন পন শব্দ বা শিস দেবার মত শব্দ হয়েছে ?
3a. Has [child's name] **ever** had wheezing or whistling in the chest at any time in the past?

☐ হ্যাঁ /Yes
☐ না / No

3b. **গত বারো মাসে** _____ (সন্ড়নের নাম) কি কখনও নি:শ্বাস নেবার সময় বুকের মধ্যে সাঁই সাঁই/ পন পন শব্দ বা শিস দেবার মত শব্দ হয়েছে ?

3b. Has [child's name] had wheezing or whistling in the chest in the last 12 months?

☐ হ্যাঁ /Yes
☐ না / No → GO TO Q. 4a

3c. সাঁই সাঁই/ পন পন শব্দ_____ (সন্ড়নের নাম) **গত বারো মাসে,** কতবার হয়েছে ?

3c. How many attacks of wheezing has [child's name] had in the last 12 months?

☐ ১ বারও না (None) ☐ ১ থেকে ৩ বার (1 to 3)
☐ ৪ থেকে ১২ বার (4 to 12) ☐ ১২ বারের অধিক (More than 12)

3d. গত বারো মাসে , এই সাঁই সাঁই/ পন পন শব্দের জন্য, _____ (সন্ড়নের নাম) মাঝ রাত্রিতে গড়ে কতবার ঘুম ভেঙ্গে যেত ?

3d. In the last 12 months, how often, on average, has [child's name]'s sleep been disturbed due to wheezing?

☐ কখনই না (Never)
☐ সপ্তাহে একরাত্রের কম (Less than one night per week)
☐ সপ্তাহে একরাত্র/ অধিক (One or more nights per week)

3e. **গত বারো মাসে ,** নিশ্বাসের এই সাঁই সাঁই/ পন পন শব্দ খুব বেশী হবার জন্য, _____ (সন্ড়নের নাম)' র কি কখনও এমন হয়েছে যে একটা বা দুটো শব্দের মাঝে নিশ্বাস নেবার জন্য থামতে হয়েছে ?

3e. In the last 12 months, has wheezing ever been severe enough to limit [child's name]'s speech to only one or two words at a time between breaths?

☐ হ্যাঁ /Yes
☐ না / No

3f. **গত বারো মাসে ,** _____ (সন্ড়নের নাম) কি দ্রুত হাটা বা সাধারণ খেলাধুলার পরে কখনও কি বুকে সাঁই সাঁই শব্দ হয়েছে ?

3f. In the last 12 months, has [child's name]'s chest sounded wheezy during or after exercise?

☐ হ্যাঁ /Yes
☐ না / No

3g. **গত বারো মাসে ,** _____ (সন্ড়নের নাম) কি দ্রুত হাটা বা সাধারণ খেলাধুলা না করেও কখনও কি বুকে নিশ্বাসের এই সাঁই সাঁই শব্দ হয়েছে ?

3g. In the last 12 months, has [child's name]'s chest sounded wheezy when he/she had not taken exercise?

☐ হ্যাঁ /Yes
☐ না / No

3h. **গত বারো মাসে,** _____ (সন্ড়নের নাম)' র কি সর্দি বা সর্দি, কাশি জ্বরের সঙ্গে বুকে সাঁই সাঁই শব্দ বা শিস দেওয়ার মতন শব্দ হয়েছিল ?

3h. In the last 12 months, has [child's name] had wheezing or whistling in the chest when he/she had a cold or flu?

☐ হ্যাঁ /Yes
☐ না / No

3i. **গত বারো মাসে,** _____ (সন্ড়ানের নাম)' র কি সর্দি বা সর্দি, কাশি জ্বর **ছাড়া বুকে** সাঁই সাঁই শব্দ বা শিস দেওয়ার মতন আওয়াজ হয়েছিল ?

3i. In the last 12 months, has [child's name] had wheezing or whistling in the chest when he/she did not have a cold or flu?

☐ হ্যাঁ /Yes
☐ না / No

3j. **গত বারো মাসে,** _____ (সন্ড়ানের নাম)' র কিসের জন্য সাঁই সাঁই শব্দ বেড়েছিল ? (Cross all that apply).

3j. In the last 12 months, what has made [child's name]'s wheezing worse? (Cross all that apply).

☐ সাবান, স্প্রে, কাপড় বা বাসন মাজার সাবান / Soaps, sprays or detergents

(Notes: Sprays include cleaning products, room freshners, insect killers & repellent, perfumes etc)

☐ সিগারেট বা বিড়ির ধুয়া/ Cigarette or bidi smoke
☐ ধুয়া / fumes
☐ ফুলের রেণু / Pollen
☐ সর্দি কাশি / Colds or flu
☐ ধুলা / Dust
☐ আবহাওয়া / Weather
☐ খাবার বা কোন পানীয় / Foods or drinks
☐ ভাবের আবেগ, উত্তেজনা / Emotion
☐ উলের কাপড়/ Wool clothing
☐ অন্য কিছু / Other things (please list below)_____

Shortness of breath

4a. _____ (সন্ড়ানের নাম) কি জীবনে কখনও শ্বাস প্রশ্বাসের কষ্টের জন্য ঘুম ভেঙ্গে গেছে ?

4a. Has [child's name] woken up with shortness of breath at any time in his/her life?

☐ হ্যাঁ /Yes
☐ না / No

4b. _____ (সন্ড়ানের নাম) কি জীবনে কখনও বুকে চাপ- চাপ ভাব' এর জন্য মাঝ রাত্রিতে ঘুম ভেঙ্গে গেছে ?

4b. Has [child's name] woken up with tightness of the chest at any time in his/her life?

☐ হ্যাঁ /Yes
☐ না / No

4c. _____ (সন্ড়নের নাম) কি তাড়াতাড়ি হাঁটতে গেলে বা একটু উচুঁতে উঠতে গেলে হাঁপিয়ে যায় ?
4c. Does [child's name] get shortness of breath walking fast or climbing up?

☐ হ্যাঁ /Yes
☐ না / No

4d. _____ (সন্ড়নের নাম) কি সমান জমিতে বা রাস্ড়য় সমবয়সীয় ছেলেমেয়েদের সঙ্গে হাঁটতে গেলে হাঁপিয়ে যায় ?
4d. Does [child's name] get shortness of breath walking with other children of his/her own age on level ground?

☐ হ্যাঁ /Yes
☐ না / No

4e. _____ (সন্ড়নের নাম) কি সাধারন ভাবে সমান জমিতে বা রাস্ড়য় হাঁটার সময় নি:শ্বাস নিতে দাঁড়িয়ে পড়ে ?
4e. Does [child's name] have to stop for breath when walking at his/her own pace on level ground?

☐ হ্যাঁ /Yes
☐ না / No

Other illnesses

5. _____ (সন্ড়নের নাম)'র কি কোনদিন এজমা / হাঁপানি বা টান ছিল ?
5. Has [child's name] ever had asthma?

☐ হ্যাঁ /Yes
☐ না / No

6a. _____ (সন্ড়নের নাম) কে কোন ডাক্তার কখনও কি বলেছে যে ওর নিউমোনিয়া হয়েছে ?
6a. Has [child's name] ever been told by any doctor that he/she has pneumonia?

☐ হ্যাঁ / Yes
☐ না / No → GO TO Q. 7a

6b. _____ (সন্ড়নের নাম) কে **গত বারো মাসে** কোন ডাক্তার কি বলেছে যে ওর নিউমোনিয়া হয়েছে ?
6b. Has [child's name] been told by the doctor that he/she has pneumonia in the past 12 months?

☐ হ্যাঁ /Yes
☐ না / No

7a. _____ (সন্ড়নের নাম) কে কোন ডাক্তার কখনও কি বলেছে যে ওর টিবি বা যক্ষা হয়েছে ?
7a. Has [child's name] ever been told by the doctor that he/she has tuberculosis?

☐ হ্যাঁ / Yes
☐ না / No → GO TO Q. 8

7b. _____ (সন্ড়নের নাম) কে **গত বারো মাসে** কোন ডাক্তার কি বলেছে যে ওর টিবি বা যক্ষা হয়েছে ?
7b. Has [child's name] been told by the doctor that he/she has tuberculosis in the past 12 months?

☐ হ্যাঁ /Yes
☐ না / No

8. **এখন কিছু প্রশ্ন করব _____ (সন্ড়নের নাম) সাধারন স্বাস্থ্য ও পারিপার্শ্বিক পরিবেশ সম্পর্কে ।**

8. Now I would like to ask you some questions about [child's name]'s general health and environment.

9a. _____ (সন্ড়নের নাম) সপ্তাহে কতবার অধিক শারীরিক পরিশ্রম (যেমন কাঠ কাটা, রিক্সা চালানো) বা খেলাধুলা (যেমন ফুটবল, ক্রিকেট, ছোয়াছুয়ি, লুকোচুরি, সাঁতার কাটা বা সাইকেল চালানো) করে ?

9b. How many times a week does [child's name] engage in vigorous physical activity (like chopping woods, pulling rickshaw etc.) and playing games like football/soccer, cricket, catching, I spy you, swimming, cycling etc.)

- ☐ কখনই না বা মাঝেসাঝে (Never or occasionally)
- ☐ সপ্তাহে একবার বা দুইবার (Once or twice per week)
- ☐ সপ্তাহে তিনবার বা বেশী (Three or more times a week)

9c. _____ (সন্ড়নের নাম) যখন ওর মায়ের / আপনার পেটে ছিল তখন বাড়িতে কি এক বা একের বেশী বেড়াল ছিল?

9c. Did you have one or more cats in your home while the mother of [child's name] was pregnant with [child's name]?

- ☐ হ্যাঁ /Yes
- ☐ না / No

9d. _____ (সন্ড়নের নাম)'র জন্মের পর থেকে এক বছর বয়স পর্যন্ত বাড়িতে কি এক বা একের বেশী বেড়াল ছিল ?

9d. Did you have one or more cats in your home during the first year of [child's name]'s life?

- ☐ হ্যাঁ /Yes
- ☐ না / No

SKIN LESIONS

THIS SECTION SHOULD ONLY BE COMPLETED AFTER THE PARTICIPANT HAS UNDERGONE THE MEDICAL EXAMINATION.
Based on the results of that examination, if the participant has no skin lesions, skip this section. If the participant has indications of skin lesions, ask the mother the following questions.

এখন কিছু প্রশ্ন করব _____ (সন্ড়নের নাম)'র শরীরের ছিটছিটে দাগ সম্পর্কে ।
I would like to ask you about [child's name]'s skin pigmentations.

1. _____ (সন্ড়নের নাম)'র শরীরের কি কোন ছিটছিটে দাগ আছে ?

1. Does he/she have pigmentation anywhere on his/her body?

- ☐ হ্যাঁ /Yes
- ☐ না / No **(Go to Q.3)**

1a. _____ (সন্ড়নের নাম)'র শরীরের প্রথম কোন বছর থেকে এই ছিটছিটে দাগ দেখা দিয়েছে ?

1a. In which year did the pigmentation changes **first** appear on [child's name] skin? |___|___|___|___| Year

1b. _____ (সন্ড়নের নাম)'র শরীরের কোন জায়গায় প্রথম এইরকম দাগ দেখা দিয়েছিল (বর্ণনা দাও) ?

1b. Where on [child's name]'s body did it **first** occur (describe) _____

1c. _____ (সন্ড়নের নাম)'র তখন বয়স কত ছিল ?

1c. How old [child's name] was then? |___|___| Years

2. _____ (সন্ড্রনের নাম) গত দুই বৎসরে কি এই ছিটছিটে দাগের কোন রকম পরিবর্তন দেখা গেছে ?

2. In the last two years, have [child's name]'s pigmentation changes improved, worsened or stayed the same?

☐ ভালোর দিকে / improved ☐ খারাপের দিকে / worsened ☐ একই রকম / stayed the same

3. _____ (সন্ড্রনের নাম)'র শরীরে কি কোন চামড়া শক্ত হবার **লক্ষণ** দেখা গেছে ?

3. Does he/she have thickening anywhere on his/her body?

☐ হ্যাঁ /Yes

☐ না / No (Skip all other question)

3a. কোন বৎসর _____ (সন্ড্রনের নাম) চামড়া শক্ত হবার **লক্ষণ** দেখা গেছে ?

3a. When did signs of skin thickening **first** appear (year)? |___|___|___|___| Year

3b. কোন বৎসর _____ (সন্ড্রনের নাম) চামড়া শক্ত হতে প্রথম লক্ষ্য করেছেন ?

3b. When did you notice the skin thickening **first** (year)? |___|___|___|___| Years

3c. শরীরের কোন জায়গায় _____ (সন্ড্রনের নাম) প্রথম চামড়া শক্ত হতে দেখা গেছে ?

3c. Where on [child's name]'s body did the thickening **first** occur (describe)?

☐ করতল (palms) ☐ পায়ের তলায় (soles)

3d. তখন _____ (সন্ড্রনের নাম)'র বয়স কত ছিল ?

How old was [child's name] then? |___|___| Years

Ending Time: |___|___|.|___|___| AM/PM

Interviewer Code: |___|___|

Interviewer Signature:_____

Appendix-5b: Respiratory Health Questionnaire (Pdf)

ACRH Study ID | A | C | R | H | | | | |

RESPIRATORY HEALTH QUESTIONNAIRE

Starting Time: |__|__|:|__|__| AM/PM **Date:** |__|__|__|__|__|__| DD/MM/YY

Respondent's relationship to participant:_____ **CID:** |__|__|__|__|__|__|__|__|__|__|

RID: |__|__|__|__|__|__|__|__|__|__|

1. এখন আমি কিছু প্রশ্ন জিজ্ঞাসা করব _____ (সন্তানের নাম) যে কারণে কাশি হয়েছে কি না সে সম্পর্কে
1. **Now I would like to ask a few questions about any coughing [child's name] might have experienced.**

1a. গত বারো মাসে, _____ (সন্তানের নাম) কি সর্দি কাশিতে বুকে কফ জমা বা কাশির সঙ্গে কফ বের হয়েছিল?
1a. **In the last 12 months, has [child's name] usually seemed congested in the chest or coughed up phlegm (mucus) when he/she had a cold?**

☐ হ্যাঁ/Yes
☐ না/No

1b. গত বারো মাসে _____ (সন্তানের নাম) কি সর্দি ছাড়া বুকে কফ জমা বা কাশির সঙ্গে কফ বের হয়েছে ?
1b. **In the last 12 months, has [child's name] usually seemed congested in the chest or coughed up phlegm (mucus) when he/she did not have a cold?**

☐ হ্যাঁ/Yes
☐ না /No → GO TO Q.2

1c. গত বারো মাসে , _____ (সন্তানের নাম) কি সপ্তাহের বেশীরভাগ দিন (সপ্তাহে ৪ বা অধিক দিন) এবং বছরের ৩ মাস, টানা বা কিছু কম বা বেশি সময়ে কফ বের হয়েছে ?
1c. **In the last 12 months, has [child's name] usually seemed congested in the chest or coughed up phlegm (mucus) on most days (4 or more days a week) for as much as 3 months of the year?**

☐ হ্যাঁ/Yes
☐ না /No → GO TO Q.2

1d. এভাবে কতদিন ধরে হচ্ছে ? **For how many years has this happened?** |__|__| বছর (Years)

2. গত বারো মাসে , _____ (সন্তানের নাম) কি রাতে ঘুমানোর সময় কি সর্দি ছাড়া শুকনো কাশি হয়েছে ?
2. **In the last 12 months, has [child's name] had a dry cough at night, apart from a cough associated with a cold or chest infection?**

☐ হ্যাঁ/Yes

☐ না /No

Wheezing

3. এখন কিছু প্রশ্ন জিজ্ঞাসা করব _____ (সন্তানের নাম) বুকে কোন শিষ শিষ/ শন শন শব্দ বা শ্বাসকষ্ট হওয়া সম্পর্কে।
3. **I would like to ask some questions about any wheezing or breathlessness [child's name] may have ever experienced.**

3a. _____ (সন্তানের নাম) কি অতীতে / জীবনে কখনও নিঃশ্বাস নেয়ার সময় বুকের মধ্যে শাঁই শাঁই/ শন শন শব্দ বা শিস নেয়ার মত শব্দ হয়েছে ?
3a. **Has [child's name] ever had wheezing or whistling in the chest at any time in the past?**

☐ হ্যাঁ/Yes
☐ না/No

3b. গত বারো মাসে _____ (সন্তানের নাম) কি কখনও নিঃশ্বাস নেয়ার সময় বুকের মধ্যে শাঁই শাঁই/ শন শন শব্দ বা শিস নেয়ার মত শব্দ হয়েছে ?
3b. **Has [child's name] had wheezing or whistling in the chest in the last 12 months?**

☐ হ্যাঁ/Yes
☐ না /No → GO TO Q. 4a

Appendix-6a: Physical Examination and Biological Samle Checklist

PHYSICAL EXAMINATION AND BIOLOGICAL SAMPLE CHECKLIST

CID: |___|___|___|___|___|___|___|___|___|

RID: |___|___|___|___|___|___|___|___|___|___|

1. Examination Information

1a. Date of Exam |_ _|_ _|_ _|_ _|_ _|_ _|_ _|
DD/MM/YY

1b. Time of Exam (write in and circle AM/PM) |___|___| . |___|___| AM/PM

2. Physical Development

One team member to record two measurements each of height and weight.

2a. Height (cm) |____|____|____|.|____|
1st Measurement

|____|____|____|.|____| 2nd
Measurement

2b. Weight (kg) |____|____|. |____|
1st Measurement

|____|____|. |____| 2nd
Measurement

3. Blood Pressure Measurement

2a. Blood pressure (Systolic/Diastolic) mmg |____|____|____|/|____|____|
1st Measurement

|____|____|____|/|____|____| 2nd
Measurement

4.1. LUNG FUNCTION MEASUREMENTS

Lung function measurements with spirometry. Primary parameters: FEV_1, FVC, FEV_1/FVC, FEF_{25-75}, FEF_{25-75}/FVC, FEF_{75}.

The EasyOne model spirometer displays, saves and can output data to a printer or PC. The readings taken will be saved and printed out.

4.1a. Spirometer Output Printed Out ☐ Yes ☐ No

4.1b. Number of Spirometry Attempts |____| Runs

4.2. Lung Symptoms and Signs

4.2a. Lung Symptoms

Cough ☐ Yes ☐ No

If yes, please specify_____

Breathing difficulty ☐ Yes ☐ No

If yes, please specify_____

4.2b. Lung Signs

Breath sound ☐ Vesicular ☐ Bronchial ☐ Vesicular with prolonged expiration ☐ Other added sound (specify)_____

4.3c. Additional Lung Symptom and Sign Observations (Specify)

_____

4.4d. Any other physical/systemic findings?

☐ Yes ☐ No

*If "yes" please specify*_____

5. Drug history

5a. Is the [child's name] taking any medication? ☐ Yes ☐ No

5b. If yes, please specify_____

6. Skin Lesions OR Skin Changes

6a. Does the participant show signs of hyperpigmentation, hypopigmentation, or both?

☐ No Pigmentation Changes ☐ Melanosis (Early Stage)
☐ Hyperpigmentation ☐ Hypopigmentation

☐ Both Occurring Side-by-Side

6b. Location of the pigmentation change (mark or write in):

☐ Chest/Torso ☐ Back
☐ Arms ☐ Legs

6c. How confident are you that [child's name]'s pigmentation changes are arsenic-induced?

☐ Definite ☐ Probable
☐ Possible ☐ Not arsenic related

6d. Does the participant show signs of keratoses on the palms or soles or both?

☐ No Keratoses ☐ Keratoses on Palms
☐ Keratoses on Soles ☐ Keratoses on Palms and Soles

6e. If participant shows signs of keratoses: Is it nodular, elevated or flat?

☐ Nodular ☐ Elevated ☐ Flat

6f. How confident are you that [child's name]'s keratoses changes are arsenic-induced?

☐ Definite ☐ Probable
☐ Possible ☐ Not arsenic related

6g. Photographs should be taken of suspected skin lesions. Were photographs taken of this participant?

☐ Yes ☐ No

6h. If no, explain why photo could not be taken.

7. Blood sample taken ☐ Yes ☐ No

If no, please specify the reasons_____

8. Urine Sample

Spot urine samples should be taken and frozen at sub-center.

8a. Was a spot void taken? ☐ Yes ☐ No

8b. Total number of urine samples from this participant (circle or write in): 1 2 3 _____

8c. Time of sample (write in and circle AM/PM): |___|___| . |___|___| AM/PM

8d. Was urine sample frozen? ☐ Yes ☐ No

8e. Result of urinary protein test (circle): 0 (nil) 1+ 2+ 3+ 4+

8f. Result of urinary glucose test (write in): _____ mg/L

8g. Was blood observed in urine? ☐ Yes ☐ No

Name of Physician_____Signature of Physician_____

Appendix-6b: Physical Examination and Biological Samle Checklist (Pdf)

Arsenic in early life and effects in childhood and Adolescence Study ID |__|__|__|

PHYSICAL EXAMINATION AND BIOLOGICAL SAMPLE CHECKLIST

CID: |__|__|__|__|__|__|__|__|__|__|

RID: |__|__|__|__|__|__|__|__|__|__|

1. Examination Information

1a. Date of Exam |_ _|_ _|_ _|_ _| DD/MM/YY

1b. Time of Exam (write in and circle AM/PM) |__|__| . |__|__| AM/PM

2. Physical Development

One team member to record two measurements each of height and weight.

2a. Height (cm) |___|___|___|.|___| 1st Measurement

|___|___|___|.|___| 2nd Measurement

2b. Weight (kg) |___|___|. |___| 1st Measurement

|___|___|. |___| 2nd Measurement

3. Blood Pressure Measurement

2a. Blood pressure (Systolic/Diastolic) mmg |___|___|___|/|___|___| 1st Measurement

|___|___|___|/|___|___| 2nd Measurement

4.1. LUNG FUNCTION MEASUREMENTS

Lung function measurements with spirometry. Primary parameters: FEV_1, FVC, FEV_1/FVC, FEF_{25-75}, FEF_{25-75}/FVC, FEF_{75}.

The EasyOne model spirometer displays, saves and can output data to a printer or PC. The readings taken will be saved and printed out.

4.1a. Spirometer Output Printed Out ☐ Yes ☐ No

4.1b. Number of Spirometry Attempts |___| Runs

4.2. Lung Symptoms and Signs

4.2a. Lung Symptoms

Cough ☐ Yes ☐ No

Appendix-7: Article published on respiratory effects by early life Arsenic exposure observed in cohort phase-1

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Chronic respiratory symptoms in children following *in utero* and early life exposure to arsenic in drinking water in Bangladesh

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Background Arsenic exposure via drinking water increases the risk of chronic respiratory disease in adults. However, information on pulmonary health effects in children after early life exposure is limited.

Methods This population-based cohort study set in rural Matlab, Bangladesh, assessed lung function and respiratory symptoms of 650 children aged 7–17 years. Children with *in utero* and early life arsenic exposure were compared with children exposed to less than 10 μg/l *in utero* and throughout childhood. Because most children drank the same water as their mother had drunk during pregnancy, we could not assess only *in utero* or only childhood exposure.

Results Children exposed *in utero* to more than 500 μg/l of arsenic were more than eight times more likely to report wheezing when not having a cold [odds ratio (OR) = 8.41, 95% confidence interval (CI): 1.66–42.6, $P < 0.01$] and more than three times more likely to report shortness of breath when walking on level ground (OR = 3.86, 95% CI: 1.09–13.7, $P = 0.02$) and when walking fast or climbing (OR = 3.19, 95% CI: 1.22–8.32, $P < 0.01$]. However, there was little evidence of reduced lung function in either exposure category.

Conclusions Children with high *in utero* and early life arsenic exposure had marked increases in several chronic respiratory symptoms, which could be due to *in utero* exposure or to early life exposure, or to both. Our findings suggest that arsenic in water has early pulmonary effects and that respiratory symptoms are a better marker of early life arsenic toxicity than changes in lung function measured by spirometry.

Keywords Arsenic, lung function, respiratory, pulmonary, *in utero*, children