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Health Effects of Arsenic Longitudinal Study (HEALS): Description of a multidisciplinary epidemiologic investigation

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Health Effects of Arsenic Longitudinal Study (HEALS), a multidisciplinary and large prospective cohort study in Araihazar, Bangladesh, was established to evaluate the effects of full-dose range arsenic (As) exposure on various health outcomes, including premalignant and malignant skin tumors, total mortality, pregnancy outcomes, and children's cognitive development. In this paper, we provide descriptions of the study methods including study design, study population, data collection, response rates, and exposure and outcome assessments. We also present characteristics of the study participants including the distribution of exposure and the prevalence of skin lesion at baseline recruitment. A total of 11,746 married men and women between 18 and 75 years of age participated in the study at baseline (a response rate of 98%) and completed a full questionnaire interview that included a food frequency questionnaire, with a response rate of 98%. Among the 98% of the participants who completed the clinical evaluation, over 90% provided blood samples and spot urine samples. Higher educational status, male gender, and presence of premalignant skin lesions were associated with an increased likelihood of providing blood and urine samples. Older participants were less likely to donate a blood sample. About one-third of the participants consumed water from a well with As concentration in each of three groups: $>100\,\mu g/l$, 25–100 $\mu g/l$, and $<25\,\mu g/l$. Average urinary As concentrations were 140 and 136 $\mu g/l$ for males and females, respectively. HEALS has several unique features, including a prospective study design, comprehensive assessments of both past and future changes in As exposure at the individual level, a large repository of biological samples, and a full dose range of As exposures in the study population. HEALS is a valuable resource for examining novel research questions on the health effects of As exposure.
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Introduction

Human health effects of arsenic (As) exposure from drinking water are a major public health issue both for the US and other countries throughout the world including Chile, Argentina, Mexico, Taiwan, Mongolia, and most recently Bangladesh and West Bengal, India.

Over 100 million people in the world, including more than three million in the US and about 50 million in Bangladesh (among 139 million in the country), are chronically exposed to As through drinking water (The British Geological Survey, 1999; EPA, 2000; EPA, 2001). Chronic exposure to naturally occurring high levels of As in drinking water has been associated with cancers of the skin, lung, bladder, liver, and kidney among As-exposed populations in different parts of the world (Brown et al., 1989; Chen and Wang, 1990; Hertz-Picciotto and Smith, 1993; Buchet and Lison, 1998; Hopenhayn-Rich et al., 1998). Arsenic exposure has also been linked to cardiovascular (ischemic heart disease, hypertension, stroke) (Chen et al., 1995, 1996; Chiou et al., 1997), endocrine (diabetes mellitus) (Lai et al., 1994; Tseng et al., 2000), and neurodevelopmental (peripheral neuropathy, cognitive development) (Tseng, 2003; Tsai et al., 2003; Wasserman et al., 2004) disorders.

Although the exact time of onset of As exposure in Bangladesh is unknown, it is suspected to have started during the 1960s and 1970s when the United Nations Children's Emergency Fund (UNICEF), in collaboration with the

1. Abbreviations: As, arsenic; HEALS, The Health Effects of Arsenic Longitudinal Study; UNICEF, The United Nations Children's Emergency Fund; GPS, geographic positional system; LDEO, Lamont–Doherty Earth Observatory; GFAA, graphite furnace atomic absorption; ICP-MS, inductively coupled plasma-mass spectrometry; CAI, cumulative arsenic index; FFQ, food frequency questionnaire; BMI, body mass index; NGO, non-governmental organization

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Bangladeshi government, started to install hand-pumped tube wells to provide pathogen-free drinking water to the population, which had long been experiencing alarmingly high incidence and mortality rates of waterborne diseases. A tube well consists of a hand pump on top of a PVC tube and filter ~5 cm in diameter that can be inserted manually into the ground to a depth of ~100 m by a small team of drillers within a day. In the late 1980s, the problem of As in drinking water and arsenical skin lesions were first documented in adjoining West Bengal, India (Chakraborty and Saha, 1987; Guha Mazumder et al., 1988). In the 1990s, it was first discovered that the population of Bangladesh had been drinking tube well water contaminated with high levels of As. This became evident when a previously unknown epidemic of skin lesions started to surface among the Bangladeshi population. Nationwide surveys have established that nearly half of the roughly 10 million tube wells serving 95% of the Bangladesh population are contaminated (The British Geological Survey, 1999; Ahsan et al., 2000; Anawar et al., 2002). Since millions of people in the country have already accrued decades of chronic exposure, a worsening epidemic of As-induced skin and other cancers in Bangladesh is almost inevitable.

Long-term studies from Taiwan showed that once chronically exposed, the exposed population continues to experience an elevated risk of cancers even several decades after the exposure has ceased (Chen et al., 1988, 1985; Chiou et al., 2001). By extrapolating the dose-specific risk estimates observed in Taiwan to the population of Bangladesh, we recently estimated that the lifetime risks of deaths due to As-induced cancers in Bangladesh are at least doubled because of the As problem (Chen and Ahsan, 2004a). Large-scale systematic epidemiologic studies are urgently needed to examine the dose-specific health effects of As exposure in the Bangladeshi population for assessing the true magnitude of the problem. Further, based on the studies in Taiwan, South America, and the US, we currently only know the health effects of As exposure for the high-dose range ($\geqslant 100\,\mu$g/l). The health effects at the lower end of the As exposure doses ($<100\,\mu$g/l) are largely unknown. Millions of individuals in both Bangladesh and the US continue to be exposed to As through drinking water in the range of $<100\,\mu$g/l; therefore, a systematic effort to understand the health effects of As in this dose range is of both scientific and regulatory interest. Lastly, most of the prospective studies conducted to date to examine the health effects of As exposure have employed ecological designs to assess the dose–response relationships. This is either because the exposure had ceased many years prior to the conduct of the study or the population drank water from multiple diverse sources, making individual-level exposure assessment extremely difficult. In Bangladesh, where the majority of the population uses a single well for their primary source of drinking water, a unique opportunity for epidemiologic studies employing individual-level exposure assessment exists.

In 2000, researchers from Columbia University and Bangladesh established the Health Effects of Arsenic Longitudinal Study (HEALS), a prospective cohort study of nearly 12,000 men and women in Araihazar, Bangladesh, to investigate the health effects of As exposure utilizing individual-level exposure assessment, with an initial focus on skin lesions and skin cancers and to establish a biorepository for future studies. In this paper, we present a description of this prospective cohort study including a description of the baseline cohort, the research resources built by the cohort study and their implications for concurrent ancillary studies as well as future observational and intervention studies within the cohort.

Methods

Overall Design and Rationale

The problem of As in drinking water in Bangladesh has both immediate and long-term ramifications from the health, earth, and social sciences perspective. Consequently, a goal in conceptualizing this study was to design an epidemiologic research study that could directly address short, intermediate, and long-term health consequences of As exposure from epidemiologic, molecular, and clinical perspectives, and that also could integrate health, earth, and social science mitigation efforts and their evaluations using health-related end points. While the absence of health and related infrastructure for building research plans was a challenge, this also provided an opportunity to establish necessary resources and infrastructure in a manner conducive to innovative and integrated approaches. In designing the study, the principal aim was to investigate the effects of As exposure on cancers (skin cancer, bladder cancer, and lung cancer), reproductive health, and children's cognitive development within a population-based cohort setting. Within the context of the prospective cohort design, a series of cross-sectional and nested case–control and case–cohort designs were planned for examining selected health outcomes (see Figure 1.). In addition, provisions for randomized clinical trials, as well as community/field intervention trials were also preserved. Finally, for ethical reasons, we have vigorously explored and implemented various mitigation strategies to reduce As exposure in this population. The designs of different components of the study are described below.

Study Area

Our goal is to examine the health effects of As, especially to evaluate the dose–response relationships between various levels of As exposure and health outcome. Therefore, we aimed to identify a population who are exposed to the full dose range of As exposure and are homogenous in terms of

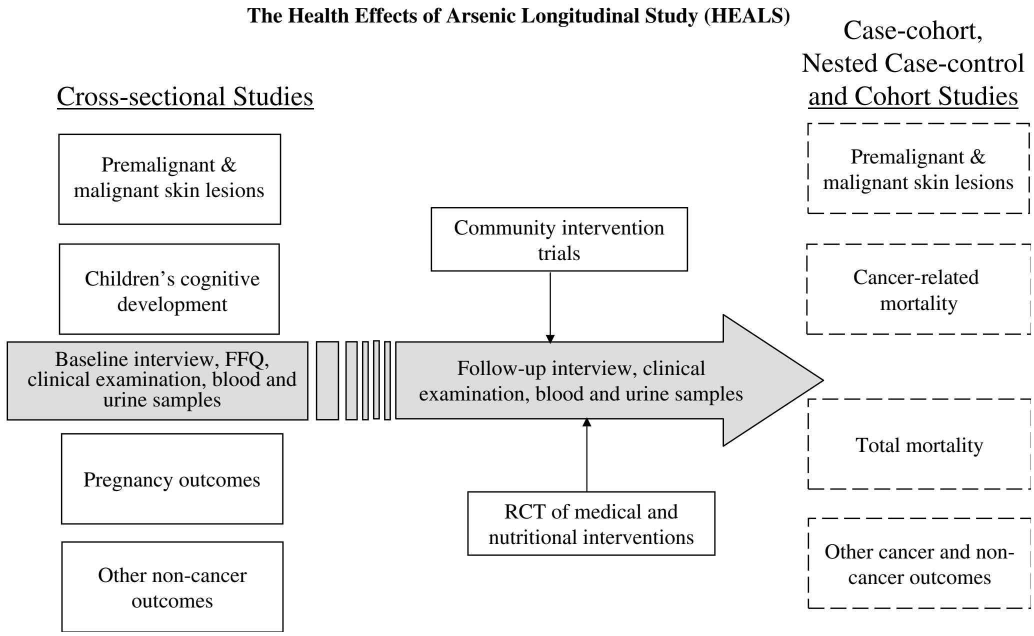

Figure 1. Design and components of HEALS.

other characteristics. With the help of the government and non-government scientists as well as other As experts in Bangladesh, we conducted a series of pilot surveys in three different regions of the country likely to meet our expected criteria. In each of the three areas, we systematically tested tube wells roughly every 150 m in a minimum of 10 consecutive wells. Based on these pilot surveys, we chose an area about 25 km southeast of the capital city Dhaka. This area was selected based on several considerations. First, this area contains a population with relatively homogenous sociocultural characteristics, while the As exposure distribution encompasses the full range between 0.1 and 864 μg/l. Second, this area had not been subject to prior As testing or other As-related research/mitigation activities. Consequently, the study population had not changed their As exposure/drinking patterns due to prior knowledge of water As concentrations. Third, the area includes approximately 70,000 residents, providing a reasonable study base as a source of at least 10,000 eligible study participants with our target characteristics. Fourth, the study area met practical considerations including presence of electricity, reasonable distance from the capital city, and good communication to and from the major hospitals and our central project office. The selected study area is located within 'Araihazar' upazilla (subdistrict)—one of the 507 upazillas in the country. Araihazar has an area of 183 km^2 and contains 12 'unions', the smallest administrative units in Bangladesh, which consist of 10–15 villages (Bangladesh Bureau of Statistics, 2004). Three unions (Brahmmandi, Dhuptara, and Araihazar Sadar) from Araihazar upazilla were included in our cohort study area.

Source Population and Study Base

Recruitment required the creation of a study base for systematically sampling cohort members based on demographic characteristics and exposure categories. In the absence of a high-quality recent census or demographic database, we decided to first enumerate the total population in the study area and ascertain their As exposure and basic sociodemographic characteristics. The identification, enumeration, and As testing of every tube well serving the study area were considered a prerequisite for creating a sampling frame for the cohort study. Since members of the same household could share more than one well for drinking water, the measurement of As in every well was required to characterize the exposure history of the population.

Pre-cohort Survey

To create a study base to recruit eligible cohort members, a pre-cohort survey of the study population was undertaken. This survey aimed to enumerate every well in the study area and its users. A trained 12-member field team carried out the survey. The field study team identified, sampled, and processed the water from well(s) for a given bari (a cluster of houses where members of an extended family reside). They determined the position of each well within 5–10 m using hand-held Global Positioning System (GPS) receivers. They also interviewed the owner of the well about the regular users of that well and collected demographic data (including name, age, gender, marital status, and spouse information) on all users. Details of the study procedures and findings for this pre-cohort survey have been published elsewhere (van Geen et al., 2002, 2003a; Parvez et al., unpublished manuscript).

Sampling Frame for Cohort Recruitment from the Source Population

A total of 65,876 Bangladeshi men and women were enumerated in the study area defined above. Name, age, gender, and address were collected as part of the pre-cohort survey (Parvez et al., unpublished manuscript). Water samples from all 5966 wells serving the study area were tested for As using laboratory-based methods (van Geen et al., 2003a). Information on other characteristics of the wells such as age and depth was also collected. A list of married couples who consumed water from a tested well was generated from the database. Potential participants from this list were produced based on the following eligibility criteria for the cohort study: (1) married male or female, (2) resident of the study area for at least 5 years, and (3) primarily drinking water from one of the 5966 study wells for at least 3 years. The reasons for targeting married couples included (1) they are more stable and less likely to move out of study area and (2) they provide opportunities to examine interindividual variability in susceptibility and effects, since members of a given married couple are more likely to share an identical source of exposure but are genetically unrelated.

Residents of the study area fulfilling the above criteria constituted the primary pool for the potential cohort study participants. This pool of subjects was categorized based on the distribution of well water As in the primary source of drinking water. The whole study area was divided into six segments and six different study teams were primarily assigned to these six segments (see below). A working list of potential participants from this pool was generated for each of the study teams for selecting and recruiting cohort participants from their designated study area segment. This list was generated for recruitment of cohort participants with several considerations: (1) we sought to recruit a cohort with a wide exposure distribution for a full dose–response relationship examination; (2) we wanted to examine dose–response relationships within low end of the As exposure distribution, that is, between 2 and 100 μg As/l; (3) we wanted to preserve study efficiency in low-level exposure and therefore we needed to recruit enough participants with well As 2–100 μg/l. Based on these considerations, we prepared the list for targeted cohort members to roughly come from three exposure groups: 1/3 from wells with $>100\,\mu$g/l As, 1/3 from wells with 25–100 μg/l As, and 1/3 from wells with $<25\,\mu$g/l As. Ultimately, we recruited 37.34% at $>100\,\mu$g/l, 30.49% at 25–100 μg/l, and 32.16% at $<25\,\mu$g/l. Wells shared by one to six participants comprised 86% of the overall HEALS participants ($N = 10{,}494$), and no more than 14 individuals were recruited from any given well.

Recruitment of Cohort Members

Using the above-mentioned list of participants generated from the pre-cohort survey, six extensively trained teams of field staff conducted the recruitment and data collection. Each field team consisted of two interviewers (one male and one female) and one field physician. Each field team followed from one end to the other within the designated segment of the study area for that team (as mentioned above) and recruited study participants using the pre-generated list of potential participants based on pre-cohort survey. The field teams were provided with information on all wells and potential participants from each well within each bari. However, the As concentrations of the wells were not known to the field team until the interview and recruitment procedures, including physical examination, were completed. A parallel information dissemination and well-labeling effort were also implemented by the Columbia University As research group, in collaboration with social scientists from School of International and Public Affairs and earth scientists from the Lamont–Doherty Earth Observatory (LDEO). Although the labeling of the wells with As values was carried out during January–March, 2001, before the majority of study participants were recruited, the village-level information dissemination and in-depth individual-level health education messages were synchronized so that interview, clinical and biospecimen data were collected before they received these systematic information dissemination and health education messages. This approach was employed to minimize potential interviewer and respondent bias.

The study team attempted to recruit married couples whenever possible. No more than three married couples were recruited from a given well. For wells with more than three eligible couples for recruitment, the study team approached the couples in the order provided in the list until three couples or six married individuals from a given well were enrolled. If one member of a couple (often working men) was absent in the house at the time of the interview, up to three revisit attempts on different days were made to recruit him or her. After three attempts or after refusal to participate, an available member of another couple was recruited. In this manner, 11,746 men and women were recruited between October 22, 2000 and May 19, 2002, including 4803 married couples, 1901 other married women, and 239 other married men.

Response Rates for Study Participation

From the pre-cohort survey, we identified 14,828 men and women eligible for recruitment into the cohort based on our study criteria. Among these individuals, 2778 participants were not at home during any of the three attempts/visits to the bari. Of the remaining 12,050 men and women who were identified and approached for enrollment, 11,746 subjects (5042 men and 6704 women) agreed to participate in the study and 294 subjects declined, yielding an overall response rate of 97.5%.

Informed Consent

After verification of identity and eligibility, each individual was explained the details of the study objectives and procedures before inviting them to participate. Since many of the people in rural Bangladesh are unable to read, the study team explained details of the study procedures and study benefits/risks in simple language. Verbal consent was obtained from each eligible respondent who agreed to participate in the study. Participants were given the option to consent with or without donating a blood/urine sample. The provisions for withdrawing from the study at any stage, even after providing consent, were also explained to enrolled participants. The study procedures were approved by the Columbia University Institutional Review Board and the Ethical Committee of the Bangladesh Medical Research Council.

Demographics and Lifestyle Factors

Following informed consent, an extensive interview was conducted to collect information on demographic and lifestyle factors. Questions for occupational uses of fertilizers, chemical dyes, and pesticides assessed current use status, duration of use, and specific product brands. Males with outdoor occupations were queried about daytime working duration and whether their bodies were covered by clothing while outside. As women in Bangladesh universally wear traditional dresses that almost completely cover the skin of their trunk, sunlight exposure of female respondents was considered minimal and therefore was not assessed in the study. Detailed information on smoking of tobacco products was also collected. While habits of cigarette and bidi (filterless homemade or locally produced cigarettes) smoking were asked together (past or current use, duration of use, age at start, and number of sticks per day), a separate set of questions were asked for hukka smoking.

Field Medical Clinic

Since the health care delivery system in rural Bangladesh is not well developed, and no systematic data on patients seen at Government Upazilla Health Centers are available for monitoring and tracking health-related events, a dedicated three-story medical clinic was built in the study area. This clinic provides comprehensive primary health care exclusively for the cohort study participants and their family members. Information on each cohort participant, including an identification card with his/her name, bari name, village name, and a unique participant identifier is maintained at the clinic. The clinic has eight examination rooms and is fully staffed and equipped. Cohort study participants and their family members are encouraged to come to the field clinic for all their healthcare needs. Since this rural population lacks basic healthcare services from government and non-government health care facilities, there is a strong motivation to seek care at the clinic. Health care provided by the clinic is also an important incentive to continuing cohort participation. In addition to the routine study visits from participants for the research study purposes, more than 75 people per day receive treatment for primary care at the field clinic.

Biological and Environmental Sample Collection, Processing, and Storage

As part of the pre-cohort survey, water samples from all 5966 tube wells were collected. The water samples were collected in 50 ml acid-washed tubes following pumping the well for 5 min. The water samples were transferred to LDEO laboratories in New York for As analyses (see below).

At recruitment, whole venous blood samples were collected in a 10 ml vacutainer tubes containing serum separator and in a separate 2 ml EDTA tube. In addition, a spot urine sample was collected in 50 ml acid-washed tubes. Both blood and urine samples were kept in portable coolers (carried by the research team) immediately after collection until processed within 2–8 h. Blood and urine samples were stored in coolers until transfer to $-20°$ Celsius freezers at the end of the day in the study office located in Dhaka city. The 10 ml tubes were spun in a table-top centrifuge to separate the cells and serum before storage in freezers. All samples were kept in a freezer until their shipment to Columbia University on dry ice within 1–2 months. Upon receipt of the shipment at Columbia University, samples were stored and kept frozen until analyzed. So, for all cohort members, whole blood as well as clot and serum fractions are stored for ongoing and future studies. All biological samples were barcoded to minimize sample-handling errors and to maintain confidentiality.

Laboratory Analyses

Tube well water As concentrations were analyzed in the Geochemistry Research Laboratory of Columbia University LDEO by graphite furnace atomic absorption (GFAA). Details of the analysis method and quality control procedures have been published elsewhere (van Geen et al., 2003a). Since the standard GFAA method had a detection limit of 5 μg/l, water samples found to have an As concentration at the limit of detection (5 μg/l) were subsequently reanalyzed by inductively coupled plasma-mass spectrometry (ICP-MS), which has a detection limit of 0.1 μg/l (Cheng et al., 2004).

Total urinary As concentration was measured in the Columbia University Trace Metals Core Laboratory by GFAA, using the Analyst 600 graphite furnace system, essentially as described (Nixon et al., 1991). This newer version of the GFAA system has a detection limit of 1 μg/l and therefore no ICP-MS analysis was required for urine samples. Arsenic levels in urine were determined with and without adjustment for urinary creatinine levels, which were analyzed by a colorimetric Sigma Diagnostics Kit (Sigma, St. Louis, MO, USA).

Exposure Assessment and Measures

Two primary measures of As exposure were explored, that is, drinking water and urinary As concentrations. In both cases, total As concentrations were measured. Additionally, an index of cumulative exposure to As was constructed based on water As concentration, water drinking pattern, and source wells over a cumulative period. This index measure, which we refer to as "cumulative As exposure index" (CAI) was calculated by multiplying total As concentration per liter of water with the product of the amount of water consumed per day (in liters) and the duration of consumption from the index well (CAI in mg $= C$ (well water As concentration, mg/l) $\times Q$ (daily consumption of well water, l/day) $\times D$ (duration of well use, days; 365.25 $\times$ duration of well use in years)). For participants using the index well as their exclusive source of drinking water, CAI calculation was straightforward. For participants who reported drinking some water from a second well, we collected information on the proportion of drinking water from each of the wells. Since all wells in the study area were tested as part of our pre-cohort survey, we were able to calculate CAI by taking into consideration both the index and the additional well(s) and their respective patterns of use (CAI in mg $= \sum C_i Q_i D$, where C_i and Q_i denote the well As concentration and daily water consumption for the ith well). Similarly, for participants who reported use of a different well as a prior drinking source (and if it was one of the 5966 wells tested in the study area), we were able to take past exposure into consideration for calculating CAI (CAI in mg $= \sum C_i Q D_i$, where C_i and D_i denote the well As concentration and duration of well use for the ith well).

While well water As concentration as an exposure measure is directly relevant from a policy and regulatory perspective, exposure assessment based on CAI is relevant from the etiologic perspective since chronic exposure is required for most of As's health effects. Urinary As concentration is often considered to reflect relatively recent exposure, but its use in large-scale population-based studies has been understudied.

Dietary Instrument and Its Validation

Nutritional factors may modify detrimental health effects of chronic exposure to inorganic As from drinking water (Mitra et al., 2004; Smith et al., 2000; Hsueh et al., 1995). However, systematic epidemiologic research with respect to the potential modifying effects of nutrition on risk of As-related diseases is lacking. Following extensive pilot and field-testing, we developed a 39-item semiquantitative food frequency questionnaire (FFQ) (Chen et al., 2004b) that was administered to cohort participants. Since the average education level of residents in the study area was low (44% without any formal education), trained interviewers completed the FFQ through in-person interviews. In addition, we completed two separate 7-day food diaries in two different seasons among 200 randomly selected cohort participants to validate the FFQ. In the validation study, trained interviewers visited participants three times a day to directly measure and record consumptions of the 39 food items listed in the FFQ. A detailed description of this FFQ instrument, as well as the results of the validation study for this instrument has been published elsewhere (Chen et al., 2004b). The goals of the FFQ instrument were to capture total energy intake and intakes of common foods and/or micronutrients. It is possible that some food items may be sources of As exposure to this population due to irrigation with As contaminated water. However, because As exposure from food to this population is likely to be negligible in comparison to As exposure from drinking water, we did not specifically design the FFQ instrument to assess dietary exposure to As.

Study Outcomes of Immediate Interest

Premalignant and Malignant Skin Tumors and Total Mortalities Nonmalignant skin lesions are much more common than skin cancers among individuals exposed to As in drinking water. Unlike skin cancer, which takes decades to develop, these lesions can appear within a few years of exposure and usually progress through stages. The typical progression starts with hyperpigmentation of the skin of the upper chest, other parts of the trunk, arms, and legs, in a 'raindrop' pattern known as 'melanosis'. Often these hypopigmented maculas become whitish rounded dots, giving rise to a pattern known as 'leucomelanosis'. In addition to these pigmentation changes, 'hyperkeratosis', presented as bilateral thickening of the palms and soles, is often seen in As-exposed individuals. Nodular protrusions develop on the hyperkeratotic skin of the palms and soles may subsequently develop ulcerations and skin cancer. The majority of the basal and squamous cell skin cancers among As-exposed individuals are thought to develop from these keratotic areas and nodules and so these are considered to be premalignant conditions (Alain et al., 1993; National Research Council (U.S.)Subcommittee on Arsenic in Drinking Water, 1999).

After extensive piloting, we established a structured protocol for the identification of skin lesions. We used an established and unique method, based on the approach used by the surgical discipline for the quantitative assessment of the extent of body surface involvement in burn patients (Demling and Way, 1994). The principle is based on dividing the whole skin surface of human body into 11 specific segments (e.g., front of arm, back of arm, face, etc.) and assigning numerical percentages to each of these segments quantitatively based on the whole body surface area as 100%. Trained physicians recorded not only the presence/absence of skin lesions in each body segment but also detailed size, shape, and extent of skin involvement. Both male and female physicians performed the examinations ensuring the best possible cooperation from both male and female respondents.

From the assessments at baseline, 714 cases of premalignant skin lesions were diagnosed. Of those, 421 (337 men and 84 women) had only melanosis while 293 (247 men and 46 women) had keratosis and/or leucomelanosis in addition to melanosis. The prevalent skin lesion cases are also being used for cross-sectional analyses of baseline HEALS data for prevalence and dose–response analyses and the findings are being published elsewhere (Ahsan et al., unpublished manuscript).

Pregnancy Outcomes A cross-sectional study was designed to examine the effects of As exposure from drinking water on pregnancy outcomes. A sample of married women, who are also participants of the parent cohort study, were selected as study subjects for this research objective. The participants' past pregnancy outcomes were examined in relation to As exposure level. Details of these study methods and findings are being published elsewhere (Factor-litvak, P., unpublished results).

Children's Cognitive Development A series of cross-sectional studies were designed to examine the effect of As exposure from drinking water on cognitive development in children. Baseline interview for female respondents included a reproductive section for information such as age and gender on their children. A total of 6504 female participants have given at least one live birth, and the average number of living children among these women was 3.3 (SD = 1.7). We identified children of the cohort participants in the database and randomly selected different groups of children for separate studies. One cross-sectional study focused on 6-year old children ($N = 234$) and another on 10-year old children ($N = 201$). Although a cohort study of children is ideal, a series of cross-sectional designs of different age groups can provide information over different age groups. The details of these cross-sectional studies examining the effects of As exposure on children's cognitive development have been published elsewhere (Wasserman et al., 2004).

In this paper, we will present the details of the description and study procedures including preliminary descriptive results for the prospective cohort study which forms the backbone of the cross-sectional studies mentioned above.

Data Analysis

The current paper reports the description of a prospective cohort study on As exposure and health effects. Baseline data from the cohort were analyzed for tabular and descriptive statistics. Means and standard deviations were calculated for variables with a continuous distribution and proportions were calculated for variables with categorical distributions. Body mass index (BMI) was computed based on weight and height measured by the interviewers. Arsenic exposures, as well as other variables measured on a continuous scale were categorized based on the distributions among total cohort members. Spearman rank correlation coefficients were calculated to assess relationships between variables with continuous distributions. All analyses were performed using SAS 8.02 for windows.

Results

Figure 2 is a map of our study area, which shows the distribution of tube wells and HEALS participants. As can be seen, there is a wide range of As concentration in wells across the study area. Some villages show a highly mixed distributed of low- and high-As wells while others contain mostly low-As or high-As wells. The cohort study participants are distributed across all segments of the study area and their As exposure distribution is not systematically different from the underlying distribution of As in all wells of the area.

A total of 11,746 participants were enrolled into the study cohort, representing 97.5% of eligible residents who were approached for recruitment into the study. All 11,746 participants who consented to participate completed the 45-min interview including a full dietary questionnaire. Among the 11,746 participants, 11,536 (98.21%) completed the clinical examination by the physician; 11,224 (95.56%) provided urine samples and 10,777 (91.75%) provided blood samples. Participants who agreed to undergo clinical examination were more likely to provide urine samples than blood samples ($P < 0.001$). The small proportion of subjects who declined clinical examination were more likely to give blood samples than the urine samples ($P < 0.001$). Overall, 10,347 (88.09%) participants provided data on both interview and clinical examination, as well as both blood and urine samples.

Table 1 presents characteristics of the cohort members who completed the baseline interview, separately for men and women. There were 5042 men and 6704 women in the cohort with mean ages 41.63 and 33.62 years, respectively. The majority of the participants were between 20 and 40 years of age. BMI ranged between 12.80 and 40.43 kg/m^2, with means of 19.43 and 20.00 kg/m^2 for men and women, respectively. The mean BMI for HEALS participants is considerably lower than the population average BMI in developed countries, but similar to that observed for rural Bangladeshi populations and populations in other developing countries (Smith et al., 2000; Hosegood and Campbell, 2003). The socioeconomic status (SES) of HEALS participants shows considerable variability. Nearly half of the study participants, especially the women, had no formal education. Nearly half of the cohort members did not own any land and nearly two-thirds lacked a TV in their household. While the majority of the men had occupations involving manual labor, almost all of the women identified themselves as homemakers.

Nearly three-fourths of men reported to have smoked regularly, with over 60% being current smokers. The prevalence of smoking was rare in women, with only $\sim$6% reporting to have ever smoked regularly. Most men reported using fertilizers and pesticides regularly for more than 5 years. Excessive sun exposure, defined as regularly working outdoors with bare body, was observed in 5.3% of male participants. Females in Bangladesh universally wear tradi-

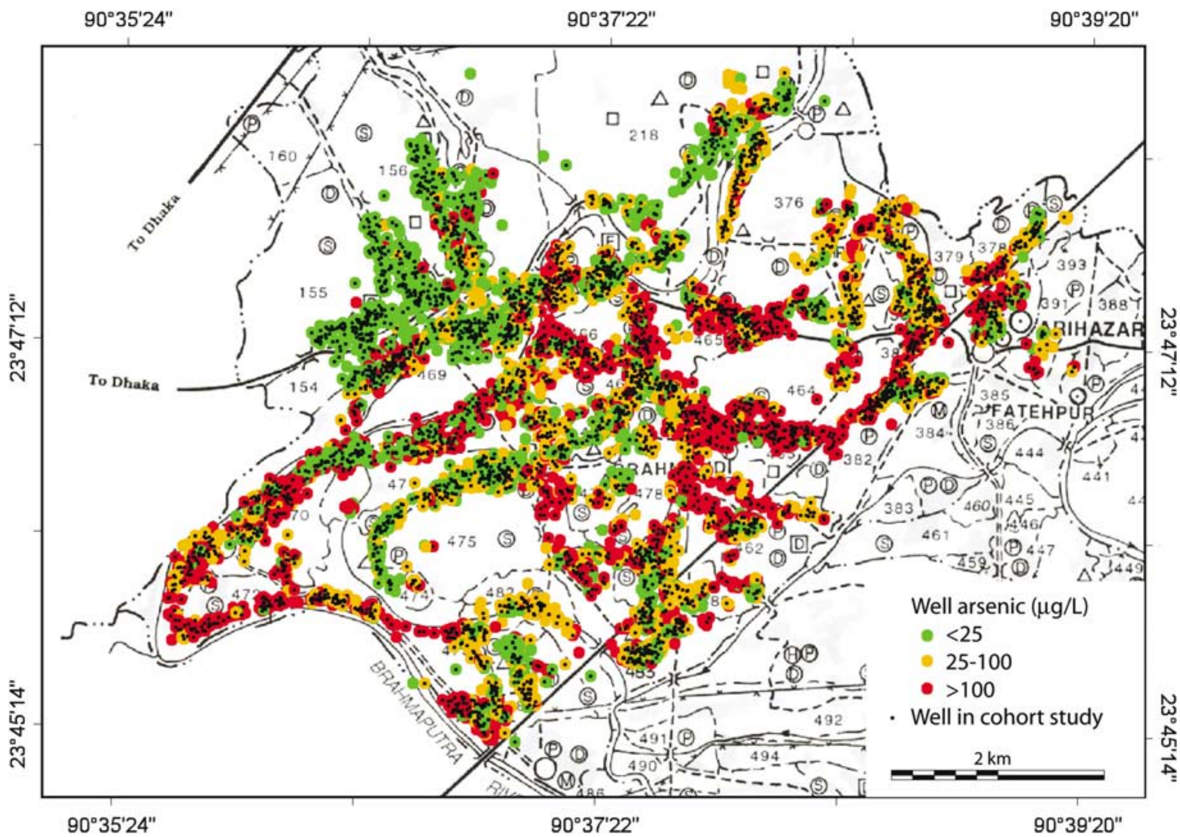

Figure 2. Geographic information system (GIS) map of HEALS study area and study wells.

tional dress, which cover most of their body; therefore, sun exposure for female respondents was considered minimal and not assessed.

Table 1 presents the distribution of As exposure for HEALS participants. As expected, there was a very wide range of As exposure based on water (0.1–864 μg/l), urine (1–2273 μg/l), and CAI (1.64–49341.62 mg) measures, and these distributions were similar for men and women. Average daily water consumption was also similar for men and women. In all, 55% of participants have been drinking water containing As $>50\,\mu$g/l—the legal limit for the country, and 76.2% have been drinking water containing As $>10\,\mu$g/l—the WHO recommended limit. However, 62.3% of the cohort members ($n=7313$ men and women) have been drinking water containing As concentrations in the range of 0–100 μg/l—a dose range of As for which the evidence for health effects is currently lacking and for which there is considerable interest among the scientific and policy-making communities. The average duration of use of wells with known As concentration was 10.0 and 8.3 years for male and female subjects, respectively.

Table 2 presents the distribution of study subjects with regard to sociodemographic and As-related variables separately for participants who completed the clinical examination, provided blood samples, or provided urine samples. Men were more likely to provide blood and urine samples but were less likely to complete the clinical examination. Older participants were less likely to provide blood samples. In general, the indicators of higher socioeconomic conditions, including higher education and land and/or television ownership, tended to be positively associated with donation of blood and urine samples. Smoking status appeared to be unrelated to donation of biological samples and completion of clinical examination. Participants with higher As exposure or those with skin lesions were more likely to donate blood samples. However, participants with higher exposure were less likely to provide urine samples.

Table 3 presents the correlation estimates among the three measures of As exposure. The relationships among the three exposure measures depend on the consistency of the pattern of water drinking of the study participants, that is, (1) whether or not a participant drank water exclusively from one index well or also from one or more additional secondary wells, (2) the amount of water consumed per day, and (3) duration of use of the well. In considering the relationship between the urinary and water-based measures, host factors related to the body's absorption, metabolism, and excretion of As are also important; these are not examined in this

Table 1. Distribution of HEALS participants by gender, sociodemographic characteristics, arsenic (As) exposure, and skin lesion status.

	Male ($N=5042$)		Female ($N=6704$)	
	No.	%	No.	%
Age (years)				
17–30	529	10.5	2427	36.2
30–39	1694	33.6	2454	36.6
40–49	1548	30.7	1454	21.7
50–59	1015	20.1	362	5.4
60–75	255	5.1	6	0.1
Mean (SD)	41.6 (9.9)		33.6 (8.8)	
Missing	1		1	
Body mass index (kg/m^2)[a]				
11.7–17.2	1078	21.9	1216	18.6
17.3–18.5	1155	23.5	1138	17.4
18.6–19.9	976	19.8	1319	20.1
19.9–22.1	887	18.0	1406	21.5
22.2–41.6	827	16.8	1465	22.4
Mean (SD)	19.4 (3.0)		20.0 (3.3)	
Missing (N)	119		160	
Education (years)				
0	2017	40.0	3220	48.0
1–5	1505	29.9	1965	29.3
6–9	724	14.4	1026	15.3
10–16	791	15.7	492	7.3
Mean (SD)	3.9 (4.1)		3.1 (3.6)	
Missing (N)	5		1	
Occupation				
Daily laborer	424	8.4	10	0.2
Farmer	708	14.0	2	0.1
Factory worker	1122	22.3	62	0.9
Other paid job	377	7.5	64	1.0
Buisness	1728	34.3	127	1.9
Unemployed	222	4.4	23	0.3
Homemakers	0	0.1	6302	94.0
Others	461	9.0	114	1.7
Owned TV				
Yes	1740	34.5	2290	34.2
Missing (N)	2		0	
Land ownership (acres)				
0	2448	48.6	3503	52.3
<1	1664	33.0	2009	30.0
1+	488	9.7	565	8.4
2–3	207	4.1	226	3.4
4+	181	3.6	189	2.8
Do not know how much	48	1.0	201	3.0
Missing (N)	6		11	
Cigarettes or bidi smoking				
Non-smokers	1286	25.5	6282	93.8
Past-smokers	609	12.1	168	2.5
Current smokers ≤10 sticks/day	1784	35.4	232	3.5
Current smokers >10 sticks/day	1359	27.0	15	0.2
Missing (N)	4		7	
Hukka smoking				
Non-smokers	3219	63.9	6513	97.2
Past-smokers	1671	33.2	162	2.4
Current users <5 times/day	76	1.5	16	0.2
Current users 5+ times/day	71	1.4	7	0.1
Missing (N)	5		6	

Table 1. *Continued*

	Male ($N = 5042$)		Female ($N = 6704$)	
	No.	%	No.	%
Use of fertilizer				
Yes	3450	68.4	1167	17.4
Years of use, Mean (SD, min, max)	11.4 (8.3, 0.5, 52)		7.1 (5.9, 0.5, 32)	
Missing (N)	0		1	
Use of pesticides				
Yes	2631	52.2	248	3.7
Years of use, Mean (SD, min, max)	8.9 (6.8, 0.5, 40)		5.3 (4.2, 1, 20)	
Missing (*N*)	2		3	
Use of dyes				
Yes	738	14.6	229	3.4
Years of use, Mean (SD, min, max)	6.4 (6.3, 0.5, 40)		5.2 (5.3, 0.5, 27)	
Missing (*N*)	2		2	
Worked outside with bare body[b]				
Yes	266	5.3	—	—
Use of well[a]				
Daily water consumption (l)				
Mean (SD, min, max)	2.9 (1.2, 0.3, 9.2)		3.1 (1.1, 0.3, 8.7)	
Years with known As exposure level				
Mean (SD, min, max)	10.0 (7.7, 0.5, 59)		8.3 (5.7, 0.5, 52)	
Well As concentration[a] *(μg/l)*				
0.1–7	1059	21.0	1386	20.7
7.1–39.0	973	19.3	1311	19.5
40.0–91.0	983	19.5	1340	20.0
92.0–179.0	1009	20.0	1353	20.2
180.0–864.0	1018	20.2	1314	19.6
Mean (SD)	102.0 (115.9)		101.1 (115.0)	
Cummulative As index (mg)[a]				
0.1–48.5	1005	20.7	1264	19.5
48.6–228.5	975	20.0	1293	20.0
228.6–587.2	956	19.6	1313	20.3
587.3–1495.7	925	19.0	1343	20.8
1495.8–9609	1010	20.7	1258	19.4
Mean (SD)	1163.4 (2398.4)		989.9 (1811.8)	
Missing (*N*)	171		233	
Total urinary As concentration (μg/l)[a]				
1–37	912	18.8	1387	21.8
37–66	948	19.5	1250	19.6
67–114	958	19.7	1290	20.3
115–205	1062	21.9	1187	18.6
206–2273	975	20.1	1255	19.7
Mean (SD)	140.0 (151.8)		136.0 (160.7)	
Missing (*N*)	187		335	
Skin lesions				
No	4355	86.4	6467	96.4
Melanosis only	337	6.7	84	1.3
Hyperkeratosis	247	4.9	46	0.7
No clinical examination	103	2.0	107	1.6

[a]Cut points were determined by quintiles of overall study population.
[b]Excessive sun exposure, defined by working outdoor with body uncovered, was assessed only for male participants.

Table 2. Distribution of sociodemographic characteristics, arsenic (As) exposure, and skin lesion status of HEALS participants by availability of blood samples, urine samples, and clinical examination data.

	Blood sample			Urine sample			Clinical examination		
	Yes (%) ($N=10777$)	No (%) ($N=969$)	ORs for Yes[a] (95% CI)	Yes (%) ($N=11224$)	No (%) ($N=522$)	ORs for Yes[a] (95% CI)	Yes (%) ($N=11536$)	No (%) ($N=210$)	ORs for Yes[a] (95% CI)
Gender									
Female	56.6	62.3	1.0	56.7	64.2	1.0	57.2	51.0	1.0
Male	43.4	37.7	1.3 (1.0–1.6)	43.3	35.8	1.4 (1.0–2.0)	42.8	49.0	0.7 (0.5–1.1)
Age (years)									
<30	25.3	24.1	1.0	25.1	27.4	1.0	25.3	20.5	1.0
30–39	35.6	31.7	1.1 (0.9–1.3)	35.2	36.6	1.2 (0.9–1.5)	35.2	38.6	0.8 (0.5–1.1)
40–49	25.4	27.4	0.8 (0.7–1.0)	25.6	24.3	1.2 (0.8–1.6)	25.6	23.3	0.9 (0.6–1.4)
50–59	11.6	13.5	0.7 (0.5–0.8)	11.8	10.0	1.6 (1.0–2.5)	11.7	14.3	0.7 (0.4–1.2)
60+	2.1	3.4	0.4 (0.3–0.6)	2.3	1.7	2.4 (0.8–7.3)	2.2	3.3	0.6 (0.3–1.4)
Education (years)									
0	43.4	58.4	1.0	44.1	56.1	1.0	44.4	55.2	1.0
1–5	29.8	26.8	1.4 (1.2–1.6)	29.7	25.9	1.4 (1.1–1.9)	29.6	24.3	1.4 (1.0–2.0)
6–9	15.4	9.7	1.8 (1.4–2.3)	15.1	11.7	1.8 (1.2–2.6)	15.0	12.4	1.3 (0.8–2.0)
10–16	11.4	5.2	2.4 (1.7–3.3)	11.1	6.3	2.4 (1.4–3.9)	11.0	8.1	1.3 (0.8–2.3)
Owned TV									
No	64.9	73.9	1.0	65.3	73.4	1.0	65.5	76.2	1.0
Yes	35.1	26.1	1.3 (1.1–1.5)	34.7	26.6	1.1 (0.9–1.4)	34.5	23.8	1.5 (1.1–2.1)
Land ownership (acres)									
0	50.1	57.6	1.0	50.6	54.6	1.0	50.6	57.1	1.0
<1	31.6	28.7	1.2 (1.0–1.3)	31.4	30.1	0.9 (0.7–1.2)	31.3	29.5	1.1 (0.8–1.6)
1+	16.2	11.4	1.3 (1.0–1.6)	16.1	10.7	1.0 (0.7–1.5)	15.9	9.1	1.7 (1.0–2.8)
Do not know how much	2.1	2.5	0.7 (0.5–1.1)	2.0	4.6	0.4 (0.2–0.7)	2.1	4.3	0.4 (0.2–0.8)
Cigarettes/bidi smoking									
Non-smokers	64.4	65.6	1.0	64.3	69.7	1.0	64.6	60.0	1.0
Past-smokers	6.7	5.7	1.3 (0.9–1.8)	6.6	6.5	0.9 (0.6–1.6)	6.6	8.1	1.0 (0.6–1.9)
Current-smokers	28.9	28.6	1.0 (0.8–1.2)	29.1	23.8	1.2 (0.8–1.8)	28.7	31.9	1.2 (0.8–1.8)
Well as concentration (μg/l)									
0.1–7	20.3	26.0	1.0	21.1	13.6	1.0	20.9	17.1	1.0
7.1–39.0	19.7	16.9	1.5 (1.3–1.9)	19.8	12.1	1.2 (0.7–1.8)	19.5	16.2	1.0 (0.6–1.6)
40.0–91.0	19.7	20.0	1.3 (1.0–1.5)	19.7	21.8	0.5 (0.4–0.8)	19.7	22.4	0.7 (0.5–1.2)
92.0–179.0	20.2	19.5	1.3 (1.1–1.6)	19.8	27.2	0.4 (0.3–0.6)	20.1	22.9	0.7 (0.5–1.1)
180.0–864.0	20.1	17.6	1.5 (1.2–1.8)	19.6	25.3	0.4 (0.3–0.6)	19.8	21.4	0.8 (0.5–1.2)
Any skin lesions									
No	92.0	93.9	1.0	93.7	59.2	1.0	—	—	—
Yes	6.4	2.9	2.4 (1.6–3.5)	6.2	2.9	1.2 (0.7–2.2)	—	—	—
No. of clinical examination	1.6	3.2	0.6 (0.4–0.8)	0.1	37.9	<0.1 (<0.1–0.01)	—	—	—

[a]Odds ratios (ORs) were adjusted for all other variables in the table. A total of 38 participants with missing values on any of the variables were excluded from calculations of adjusted odds ratios.

paper. Consistent with our expectation, among cohort participants who drank water exclusively from one index well, the relationships among all three measures were very strong, with correlation coefficients ranging between 0.64 and 0.85. Duration of well use and daily water consumption were not associated with either well water or urinary As concentrations. Among participants who consumed water from multiple wells, correlations among As exposure measures were similar, but the correlations between urinary As and the other two As exposure measures were weaker.

Table 3. Correlation coefficients and R^2 (in parentheses)[c] among different arsenic (As) exposure measures and related variables For HEALS participants consuming water primarily from one index well

As exposure measures and related variables	Cumulative As index (CAI)	Urinary creatinine-adjusted As (μg/g creatinine)	Urinary As (μg/l)	Well As concentration (μg/l)	Duration of well use (years)	Daily water consumption (l/day)
Water consumption mainly from the index well (N = 9383)[a]						
Cumulative As index (mg)	1.00	—	—	—	—	—
Urinary creatinine-adjusted As (μg/g creatinine)	0.66 (0.43)	1.00	—	—	—	—
Urinary As concentration (μg/l)	0.54 (0.29)	0.63 (0.42)	1.00	—	—	—
Well As concentration (μg/l)	0.85 (0.79)	0.73 (0.51)	0.62 (0.36)	1.00	—	—
Duration of well use (years)	0.45 (0.17)	0.06 (0.00)[d]	0.05 (0.00)[d]	0.08 (0.00)[d]	1.00	—
Daily water consumption (l/day)	0.16 (0.03)	0.06 (0.00)[d]	−0.05 (0.00)[d]	−0.04 (0.00)[d]	0.02* (0.00)[d]	1.00
Water consumption from multiple wells (N = 1455)[b]						
Cumulative As index (mg)	1.00	—	—	—	—	—
Urinary creatinine-adjusted As (μg/g creatinine)	0.46 (0.40)	1.00	—	—	—	—
Urinary As concentration (μg/l)	0.32 (0.26)	0.59 (0.41)	1.00	—	—	—
Well As concentration (μg/l)	0.77 (0.77)	0.43 (0.46)	0.35 (0.33)	1.00	—	—
Duration of well use (years)	0.41 (0.17)	0.04** (0.00)[d]	<0.01** (0.00)[d]	−0.02** (0.00)[d]	1.00	—
Daily water consumption (l/day)	0.20 (0.03)	0.09 (0.00)[d]	−0.05 (0.00)[d]	−0.03** (0.00)[d]	0.07 (0.00)[d]	1.00

*P-value between 0.05 and 0.10.
**P-value >0.10.
All other P-values were <0.05.
[a]Values for all the As exposure measures and related variables were available for 9383 participants among the 10,146 participants who consumed water mainly from the index well.
[b]Values for all the As exposure measures and related variables were available for 1455 participants among the 1600 participants consumed water from multiple wells.
[c]R^2 were computed by performing linear regression models with log-transformed values of pairwise As exposure variables.
[d]R^2 <0.005.

Discussion

Epidemiologic studies that seek to examine ! exposure and health effects are challenging in developing countries. In Bangladesh, where about 50 million people have been chronically exposed to drinking water with As concentration exceeding the WHO standard (10 μg/l) (The British Geological Survey, 1999; EPA, 2000; EPA, 2001), a systematic assessment of As exposure and its health effects is required for preventive and policy measures. Through collaborations with local researchers, we established HEALS, a prospective cohort study to work toward this goal. We report our methods, study design, and a description of study participants including As exposure characteristics and other relevant factors and potential covariates in this paper.

The current study carries several unique features and advantages that are worth highlighting and discussing. First, the study demonstrates the feasibility of conducting large-scale methodologically rigorous epidemiologic cohort studies in a developing country setting. Although a large amount of preparatory work was necessary to establish a sampling frame for subject recruitment, the sampling frame has provided an advantage for a cohort study. Data collection will be helpful for not only examining and ascertaining health effects in the same population for future studies but also to provide infrastructure for the general health needs of the local population. As a result of the establishment of the study procedures for the pre-cohort and cohort studies, as well as the creation of the study clinic, the study participants have benefited from an increased awareness of the detrimental health effects of As. Furthermore, the study clinic has become a valued resource for the study participants, contributing to their overall general health.

Second, although the carcinogenic effects of As are well established at water As concentrations >100 μg/l, the cancer risk in the low-dose range has not been well characterized. Moreover, the noncarcinogenic effects of As have been much less studied. In the HEALS cohort, 60% of the participating members are exposed to As concentrations in the 1–100 μg/l dose range. This provides us with a unique opportunity to assess the health effects of As at the low-dose range, which is directly relevant for millions of people around the world. Indeed, this study's inclusion of a large number of people exposed to <10 μg/l provides a sound anchor for comparisons.

Third, HEALS will be the first prospective cohort study where cohort members were exposed at the time of recruitment and both exposure and outcome measures are evaluated at the level of the individual. Fourth, HEALS provides a platform for other concurrent and subsequent epidemiologic, clinical, and laboratory studies as well as avenues for evaluating biomedical, nutritional, and other interventions and programs. The cohort consists of mainly adult married couples among whom the effects of As, especially cancer, cardiovascular diseases, and other chronic effects, can be examined prospectively. The children of these married couples provide another potential cohort population for examining the effects of As in children. Cross-sectional studies among these children have already answered novel research questions (Wasserman et al., 2004). The women of reproductive age within the cohort (female cohort members aged 15–45 years) comprise a unique subcohort of potential pregnant mothers for examining effects of *in utero* exposure on pregnancy outcomes. Extensive data on As exposure and relevant covariates also allowed *adhoc* pilot studies (Hafeman et al., 2005a, b). In addition, the prospective nature of this study provides an inherent opportunity to evaluate a whole range of mitigation options (biomedical and public health) for tackling this massive problem. Although unusual in an observational study, on ethical grounds, we decided *a priori* to institute public health measures to reduce As exposure among cohort participants (van Geen et al., 2002, 2003b). We devised different public health, health education, and information dissemination interventions and will evaluate their effectiveness prospectively. Urinary As will also serve as an outcome for evaluating the effectiveness of the various mitigation strategies. Another intervention research opportunity arises from the large number of premalignant skin lesion cases identified during baseline recruitment and follow-up of the 11,746 cohort members. Based on promising results from pilot intervention trials (Verret et al., 2005) such cases are being recruited as part of larger clinical trials to evaluate effectiveness of biomedical and/or nutritional intervention for preventing progression of premalignant skin lesions to skin cancer. Additionally, we developed a dietary instrument for capturing nutritional data for a typical Bangladeshi diet in our cohort, one of the first validated FFQs in Bangladesh for epidemiologic studies. A number of research questions in nutritional epidemiology can be addressed using these data.

Although large nation-wide well surveys based on field test kits have been conducted in Bangladesh (The British Geological Survey, 1999), precise estimates of the As exposure distribution based on high-quality laboratory-based assessments are currently lacking for the Bangladeshi population. Although we recruited cohort participants who met certain eligibility criteria from the general population, the exposure distribution in our participating cohort members is not different than the overall population in our study area. The exposure distribution in the general population of study area has been published previously (Chen and Ahsan, 2004a). The representativeness of the exposure distribution allowed us to estimate the risk of internal cancer mortality in the Bangladesh population by extrapolation and using effect estimates from studies in Taiwan (Chen and Ahsan, 2004a).

In HEALS, we found that male respondents were more likely to provide biological samples. This is possibly due to the higher disease burden in males or other cultural differences between men and women. As expected, participants with higher SES were more likely to provide biological samples compared with individuals of lower SES. Participants with higher SES may be more cooperative because they are more knowledgeable about benefits of modern health care. We also found that older participants were more likely to provide urine samples but less likely to provide blood samples. The implications of these findings are important for future studies of the health effects of As in this cohort, especially for hypotheses involving biological measures. Descriptive measures, that is, the prevalence of biological measures in the overall cohort, would be potentially different than the true prevalence in the underlying population because of the association of unavailability of biological samples with certain characteristics of cohort participants (e.g., age and gender). However, since only a small proportion of study participants did not provide biological specimens, the impact on the generalizability of findings in future studies involving biological measures is unlikely to be a major problem. The internal validity of findings from dose–response analyses across different exposure groups within the study population will also be unlikely to be affected. Nevertheless, in future studies within HEALS, each of the factors associated with biological samples donation observed in this study will have to be carefully examined as a potential confounder and adjusted for depending on the nature of the specific hypothesis tested.

A higher proportion of women participated in HEALS than men. This was mainly because men were more likely to be at work during visits by cohort study team. The differences in average age and duration of well use between men and women in the study may reflect the fact that women tend to marry in to men's houses from other area and that wives are usually younger than husbands. The higher proportion of female participants with biological samples would enhance the statistical power for examining As-induced premalignant and malignant conditions in women.

We found that exposure measures based on water and urine were most strongly correlated for participants who drank water from one tube well and whose drinking pattern characteristics remained relatively unchanged during the duration of tube well use. In the future, although the same three exposure measures (i.e., water As, urinary As, and CAI) will be measured at all follow-up visits, the relative utility of one exposure measure *versus* another will be an important issue in assessing dose–response relationships between As exposure and health outcomes. For example, As exposure based on water concentration can be a reasonable measure if a single well is used as the primary source of drinking water. If multiple wells are used, As concentration in the index well captures exposure to the extent that the amount of drinking water from that tube well contributes to the total water consumed from all wells. Urinary As, being a measure of total body burden of As, reflects an accurate measure of recent exposure irrespective of the number of wells from which a participant may drink water. Since health promotion is a goal of our work, as well as the objective of other government and non-governmental organization (NGO) efforts, it is likely that cohort participants will change their drinking water sources, especially individuals who were exposed to high levels of As at baseline. Therefore, looking ahead, exposure measures based on water As concentration from the current well will become less reflective of cumulative exposure as sources of drinking water become diverse. Repeated measures of urinary As, reflecting total As burden over time, will likely remain a more valid measure of As exposure. The CAI, which includes a component for duration of exposure compared with well water As concentration, would also remain a reliable measure for assessing health affects of chronic As exposure for subjects whose consumption patterns can be well characterized. If we successfully track the changes in drinking water sources accurately, then CAI will remain a valid and preferred measure for assessing cumulative As exposure and its health effects. If however, the tracking of changes in drinking water sources and other relevant information, such as duration of use, becomes problematic or not feasible, the CAI will become a less reliable measure and we may have to rely on urinary As for assessment and tracking changes of exposure in all future studies of the health effects of As exposure in this cohort as well as other studies conducted in Bangladesh.

In conclusion, As is clearly a major public health problem in Bangladesh. HEALS, the only prospective cohort study based on individual-level exposure assessment in Bangladesh (and largest in the world), will emerge as a valuable research resource for examining novel scientific hypotheses, prevention-related research questions, and also for evaluating policy interventions.

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