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Investigation of Association of the Arsenic Exposure with Inflammatory, Adhesion and Angiogenic Molecules: A Cross-Sectional Study in Bangladesh

Rahman, Md. Mashiur

University of Rajshahi

PhD Thesis

Investigation of Association of the Arsenic Exposure with Inflammatory, Adhesion and Angiogenic Molecules: A Cross-Sectional Study in Bangladesh

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Md. Mashiur Rahman

Roll No. 11322

Registration No. 1528

Session: 2011-2012

Department of Biochemistry and Molecular Biology
University of Rajshahi, Rajshai -6205, Bangladesh

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Certificate

I certify that the thesis entitled "**Investigation of Association of the Arsenic Exposure with Inflammatory, Adhesion and Angiogenic Molecules: A Cross-Sectional Study in Bangladesh**" submitted by Mr. Md. Mashiur Rahman, incorporates the original research work carried out by him in the Department of Biochemistry and Molecular Biology, University of Rajshahi, Bangladesh under my supervision. I am forwarding his thesis being submitted for the award of the degree of Doctor of Philosophy of the University of Rajshahi. This work has not been submitted previously anywhere for the awards of any degree.

(Dr. Md. Khaled Hossain)
Professor and Supervisor
Department of Biochemistry and Molecular Biology
University of Rajshahi, Bangladesh

Declaration

I hereby declare that the material embodied in this entitle "**Investigation of Association of the Arsenic Exposure with Inflammatory, Adhesion and Angiogenic Molecules: A Cross-Sectional Study in Bangladesh**" prepared for submission in the Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi-6205, Bangladesh, for the degree of Doctor of Philosophy. The work contained in this thesis is original and have not been previously submitted anywhere for the awards of any degree.

(Md. Mashiur Rahman)

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Abstract

Arsenic is an environmental pollutant and a well established human carcinogen that causes a variety of health hazards affecting millions of people in the world. Cardiovascular diseases (CVDs) and cancer are the leading causes of arsenic-related morbidity and mortality worldwide. Atherosclerosis is the main event of CVDs, and angiogenesis is involved in the pathological development of cancer and CVDs. There are several circulating inflammatory, adhesion and angiogenic molecules that are involved in atherosclerosis and angiogenesis. Among them, C-reactive protein (CRP) as an inflammatory molecule, intercellular adhesion molecule-1(ICAM-1) and vascular cell adhesion molecule-1(VCAM-1) are implicated in the development of atherosclerotic plaque, and vascular endothelial growth factor (VEGF) is involved in angiogenesis. Although arsenic exposure is associated with CVDs and cancer, the biochemical events underlying the arsenic-induced CVDs and cancer have not yet been fully documented. Therefore, this study has been designed to investigate the relationships of arsenic exposure with the circulating inflammatory, adhesion and angiogenic molecules related to CVDs and cancer. Non-endemic human subjects were selected from a village in the northern area of Bangladesh with no history of arsenic exposure, and arsenic-endemic subjects were selected from several villages in the north-west region of Bangladesh. Arsenic concentrations in water, hair and nails were measured by Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) and circulating levels of CRP, ICAM-1, VCAM-1 and VEGF were quantified using respective enzyme-linked immunosorbent assay kits through micro plate reader. Significant positive correlations were found between arsenic exposure metrics (water, hair and nails) and circulating levels of CRP, ICAM-1, VCAM-1 and VEGF. All these molecules showed dose-response relationship with arsenic exposure metrics. Further, the associations of CRP, ICAM-1, VCAM-1 and VEGF with arsenic exposure metrics were significant even adjusting for relevant covariates. Therefore, the significant positive correlations and dose-response relationships of circulating inflammatory, adhesion and angiogenic molecules with arsenic exposure metrics suggest the possible biochemical events of arsenic-induced CVDs and cancer.

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List of acronyms, abbreviations, and symbols

ANOVA	One-way Analysis of Variance
As	Arsenic
AS3MT	Arsenic methyltransferase
BFD	Black foot disease
BGS	British Geological Survey
BMI	Body Mass Index
CI	Confidence interval
CRM	Certified reference material
CRP	C-reactive protein
CVDs	Cardiovascular diseases
DBP	Diastolic blood pressure
DMA	Dimethylarsinic acid
DPHE	Department of Public Health Engineering
EDTA	Ethylenediamine-tetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
EPA	Environmental Protection Agency
HDL-C	High density lipoprotein cholesterol
iAs	Inorganic arsenic
IARC	International Agency for Research on Cancer
ICAM-1	Intercellular adhesion molecule-1
ICP-MS	Inductively Coupled Plasma Mass Spectroscopy
IL	Interleukine
mg	milligram

MMA	Monomethylarsonic acid
NMIJ	National Institute of Advanced Industrial Science and Technology, Japan
NO	Nitric oxide
ng	Nano gram
Ox-LDL	Oxidized low density lipoprotein
pg	Pico-gram
ROS	Reactive oxygen species
SAM	*S*-adenosylmethionine
SBP	Systolic blood pressure
SD	Standard deviation
SE	Standard error
SOES	School of Environmental Studies
SPSS	Statistical Packages for Social Sciences
TNF	Tumor necrosis factor
TWAS	the academy of sciences for the developing world
UNICEF	United Nations Children's Fund
USA	United States of America
VCAM-1	Vascular cell adhesion molecule-1
VEGF	Vascular endothelial growth factor
WHO	World Health Organization
µg	Microgram
8-OH-G	8-hydroxyguanine

Dedicated to my parents.....

Introduction

1.1 Background

Arsenic is a toxic metalloid widely present in food, soil, air, and water. People are generally exposed to arsenic through drinking of arsenic contaminated groundwater and consumption of rice and vegetables irrigated by contaminated groundwater. Occupational exposure to arsenic is also occurred in mining, pesticide, glass, and pharmaceuticals industries. Drinking of groundwater is considered a major source of arsenic to the people living in arsenic affected areas in some parts of the world (Meharg, 2004; Mondal and Polya, 2008; Rahman et al., 2008). Rice grown in arsenic affected areas where groundwater is extensively used for irrigation become second major exposure route of arsenic (Brammer, 2009; Brammer and Ravenscroft, 2009; Meharg, 2004; Mondal and Polya, 2008; Roy chowdhury, 2008). On the other hand arsenic exposure from air and soil are minimal. Arsenic can exist in both organic and inorganic forms in the environment. Generally arsenic is found as organic form in different types of foods that are less toxic than the inorganic arsenic (Duxbury et al., 2003; Meharg and Rahman 2003; Sun et al., 2008). However, recent report suggests that many food items from the badly affected areas also contain inorganic form of arsenic (Meharg et al., 2008). Most poisonous form of inorganic arsenic is found in drinking water (IARC, 2004; Zheng et al., 2004). Drinking water with elevated levels of inorganic arsenic is a global health concern. Bangladesh has been grappling with the largest mass poisoning of a population in history because of the contamination of drinking water by inorganic arsenic greater than the permissive limit (10µg/L) for the arsenic-endemic people set by World Health Organization (WHO). Chronic exposure to high levels arsenic is associated with dermatitis, cardiovascular diseases (CVDs), multi-side cancers, diabetes mellitus, chronic bronchitis, immune disorders, peripheral neuropathy, liver damage, renal failure, adverse reproductive outcomes, hematological effects, and other ailments (Ali et al., 2010; Argos et al., 2010; Chen et al., 2007; Mazumder et al., 1998, 2000, 2005; Meliker et al., 2007; Mumford et al., 2007; Tapio and Grosche 2006; Vahidnia et al., 2008; Wang et al., 2002). Therefore, arsenic toxicity along with the adverse health effects has also created social and economical problems.

1.2 Physico-chemical properties of arsenic

Arsenic is a metalloid has properties of both metals and non-metals. It is a group V element with symbol As, atomic number 33 and weight 74.92. Arsenic primarily exists at four different valence states +5, +3, 0 and –3. Elemental arsenic has a valence state of 0. Arsine and arsenides have a valence of–3. Arsenic is found to exist in combination with oxygen, hydrogen, chlorine, sulphur, different metals, and also as a pure elemental crystal. Arsenic can exist as powder, amorphous or vitreous forms. Elemental arsenic has a specific gravity of 5.73 sublimes at 615°C and has a very low vapour pressure of 1 mm Hg at 373°C. Many of the inorganic arsenic compounds occur as white, odourless solids with specific gravities ranging from about 1.9 to more than 5. Elemental arsenic is insoluble in water. Both organic and inorganic arsenic species can dissolve in water. Monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) are the major form of organic arsenic (Tamaki and Frankenberger, 1992). Inorganic forms of arsenic are more toxic than organic form. The trivalent form of inorganic arsenic (As^{III}, called arsenite) and the pentavalent forms (As^{V}, called arsenate) are the principal inorganic species which tend to be more prevalent in water than the organic arsenic species (Ferguson and Gavis, 1972). Under reducing conditions arsenite is the dominant form; whereas arsenate is generally the more stable form in oxygenized environments (NRC, 1999). When heated to decompose, arsenic compounds emit toxic arsenic fumes (HSDB, 2003). Arsenic trioxide, the most common arsenic compound in commerce, melts at 312°C and boils at 465°C. Arsenic does react with hot acids to form arsenous acid (H_3AsO_3) or arsenic acid (H_3AsO_4).

Table 1.1 Chemical nature of arsenic at a glance

Atomic number	33
Atomic mass	74.922 g/mol
Electronegativity according to Pauling	2.0
Density	5.7 g/cm^3 at 14°C
Melting point	814 °C (36 atm)
Boiling point	615 °C (sublimation)
Atomic radius	0.139 nm
Oxidation states	-3, +3, +5
Key isotope	^{75}As
Electronic shell	[Ar] 3d^{10} 4s^2 4p^3
Energy of first ionization	947 kJ/mol
Energy of second ionization	1798 kJ/mol
Energy of third ionization	2736 kJ/mol
Standard potential	- 0.3 V (As^{3+}/ As)
Discovered by	Albertus Magnus

Source: [Lenntech, Netherlands: Alumni from the Technical University of Delft. Available at: http://www.lenntech.com/periodic/elements/as.htm]

1.3 Occurrence and exposure rout of arsenic

Arsenic is a naturally occurring element found throughout the environment. It is released into the environment from both natural and man-made sources. It occurs in more than 245 minerals. It ranks 20th in natural abundance, 14th in seawater and 12th in the human body (Mandal and Suzuki, 2002). People are exposed to arsenic through water, food, air, occupation and other sources. It has widespread use in agriculture, medicine, ceramic and electrical industries (Nriagu and Azcue, 1990). The sources from where arsenic enters into environment are mentioned bellow:

1.3.1 Natural sources of environmental arsenic

Arsenic is distributed ubiquitously throughout in the earth crusts, soil, sediments, water, air and living organisms. The major sources of naturally occurring arsenic are as follows.

1.3.1.1 Earth crusts

Arsenic is most abundantly present in earth crust and the average concentration of arsenic in different type of rock (igneous rocks: a crystalline solids which form directly from the cooling of magma and sedimentary rocks: a thin veneer of loose sediment that cover the earth crust mostly composed of igneous rock) is 2 mg/kg. Arsenic occurs from 0.5 to 2.5 mg/kg in most rocks, and higher concentrations were found in finer-grained argillaceous sediments and phosphorites (Kabata-Pendias and Pendias, 1984). Arsenic is concentrated in some reducing marine sediment especially those associated with gold mineralization up to 3000 mg/kg. It is also concentrated with iron hydroxides and sulfides in sedimentary rocks. Iron deposits, sedimentary iron ores and manganese nodules were rich in arsenic. Arsenic naturally occurs in over 200 different mineral forms, of which approximately 60% are arsenates, 20% sulfides and sulfosalts and the remaining 20% includes arsenides, arsenites, oxides, silicates and elemental arsenic (Onishi, 1969). Arsenic in its most recoverable form is found in various types of metalliferous deposits. The major deposits of this type are categorized into seven major groups. It is common in iron pyrite, galena, chalcopyrite and less common in sphalerite (Goldschmidt, 1954). The most common arsenic mineral is arsenopyrite.

1.3.1.2 Soil and sediment

Arsenic is found in the soils of various countries range from 0.1 to 50 mg/kg and mean 5 mg/kg, but varies considerably among geographic regions (Colbourn et al., 1975; Garelick et al., 2008; Mandal and Suzuki, 2002; Vinogradov, 1959). Concentrations of arsenic in soils are higher than those in rocks and lowest in sandy soils and those derived from granites, whereas larger concentrations are found in alluvial and organic soils (Kabata-Pendias and Pendias, 1984; Peterson et al., 1981). Arsenic concentrations in uncontaminated soil are generally in the range between 0.2–40 mg/kg (WHO, 1981). The average arsenic content in agricultural land in Bangladesh varied from 4 to 8 mg/kg but it rose up to 83 mg/kg due to continuous use of contaminated irrigation water (Ullah, 1998). Arsenic and its compounds are mobile in the environment. Weathering of rocks converts arsenic sulfides to arsenic trioxide, which enters the arsenic cycle as dust or by dissolution in rain, rivers or groundwater.

1.3.1.3 Water

Groundwater is the major source of arsenic and humans are generally exposed to arsenic through contaminated drinking water. Arsenic can inter water supplies from natural deposits which then enter into the food chain, causing widespread distribution throughout the plant and animal kingdoms. The maximum permissible concentration of arsenic in drinking water is 50 µg/L and recommended value is 10 µg/L by EPA and WHO (EPA, 1975; WHO, 2001). Many study reported the presence of arsenic in drinking water exceeding WHO recommended maximum levels (50µg/L). Arsenic is found to exist in water both organic and inorganic form. Inorganic species of arsenic is more dominant in water. Under oxidation state pentavalent (As^{V}) form is more dominant, whereas in reduced environment trivalent (As^{III}) form of inorganic arsenic is more stable.

1.3.1.4 Food

Food is another important source of human exposure to arsenic. Rice is staple food in many areas of the world (Meharg and Zha, 2012). Rice and vegetables were found to contain higher amount of arsenic in arsenic-affected areas like Bangladesh because of excessive use of arsenic-contaminated groundwater for irrigation (Duxbury et al., 2003; Meharg and Rahman 2003). Toxic inorganic arsenic is present relatively high proportion in rice with increased bioavailabilities and bioaccessibilities (Juhasz et al., 2006; Meharg and Raab, 2010; Trenary et al., 2012). Organic arsenic such as arsenobetaine, arsenocholine, arsenosugars, tetramethylarsonium salts, and arsenic-containing lipids have been found in high amount in seafood and marine organisms, although some of these compounds have also been found in terrestrial species (Francesconi and Edmonds, 1997; Grotti et al., 2008). These arsenic derivatives are not acutely toxic because of their low biological reactivity and their rapid excretion in urine. Arsenic concentrations in seafood amount 2.4–16.7 mg/kg in marine fish, 3.5 mg/kg in mussels and more than 100 mg/kg in certain crustaceans (Buchet et al., 1984; Ishinishi et al., 1977). Appreciable amount of trivalent inorganic arsenic have been observed in wine made from grapes on which arsenic containing pesticide sprayed (Hughes et al., 1994). The amount of ingested arsenic via foods mainly depends on the seafood in the diet especially where seafood consumption rate is very high. It has been reported that Japanese population intake more amount of

arsenic than in Europe and the United States because of their diet contain larger amount of seafood. The diet in Japan was found to contain 5.7–17% inorganic arsenic, 1.1–3.6% monomethylarsonate (MMA), 6.6–27% dimethylarsinate (DMA) and 47.9–75.2% arsenobetaine (Yamauchi and Fowler, 1994).

1.3.1.5 Air

In the atmosphere, arsenic exists as particulate matter, mostly less than 2 µm in diameter. It is usually present in air as a mixture of arsenite and arsenate. People are generally exposed to arsenic through air is very low and normally arsenic concentrations in air ranges from 0.4 to 30 ng/m^3 and less than 1% of total arsenic exposure (WHO, 1996). According to US EPA the estimated average national exposure in the U.S. is at 6 ng/m^3 arsenic. The amount of arsenic inhaled per day is about 50 ng or less (assuming that about 20 m^3 of air is inhaled per day) in unpolluted areas (WHO, 1981). The daily respiratory intake of arsenic is approximately 120 ng of which 30 ng would be absorbed (Zuane, 1990). Typical arsenic levels for the European region are currently quoted as being between 0.2 and 1.5 ng/m^3 in rural areas, 0.5 and 3 ng/m^3 in urban areas and no more than 50 ng/m^3 in industrial areas (DG Environment, 2000).

1.3.2 Anthropogenic source of arsenic

Elemental arsenic is produced commercially from arsenic trioxide. Arsenic trioxide is a by-product of metal smelting operations. About 70% of the world production of arsenic is used in timber treatment, 22% in agricultural chemicals and the remainder in glass, pharmaceuticals and metallic alloys [IPCS (International Programme on Chemical Safety)]. Mining, metal smelting and burning of fossil fuels are the major industrial processes that contribute to arsenic contamination of air, water and soil. The use of arsenic-containing pesticides in the past has left large areas of agricultural land contaminated. The use of arsenic in the preservation of timber has also led to contamination of the environment. In addition, the use of arsenic-contaminated groundwater for irrigation leads to widespread contamination of land and additional exposure to human and livestock via food all over the world (Kile et al., 2007; Lindberg et al., 2007; Meharg and Rahman, 2003).

1.4 Environmental transport and distribution of arsenic

In the atmosphere, arsenic is emitted by high-temperature processes such as coal-fired power generation plants, burning vegetation and volcanism. Primarily, arsenic in the atmosphere is released as As_2O_3 and exists mainly adsorbed on particulate matter. These particles are then dispersed through wind and returned to the earth by wet or dry deposition.

In soils or sediments arsines released from microbial sources undergo oxidation in the air and reconverting the arsenic to non-volatile forms that settle back to the ground. The water soluble forms of arsenic include arsenate, arsenite, methylarsonic acid (MMA) and dimethylarsenic acid (DMA). Almost all arsenic present in well-oxigenated water and sediments is in more stable pentavalent state (arsenate). Depending on the redox potential, pH, and biological processes, some arsenic species can interchange their oxidation state. There is also affinity of some arsenic species for clay mineral surfaces and organic matter which lead to affect their environmental behaviour. Weathered rock and soil may be transported by wind or water erosion. Many arsenic compounds tend to adsorb to soils and leaching usually results in transportation over only short distances in soil. There are three major modes of arsenic biotransformation in arsenic cycle found to occur in the environment: redox transformation between arsenite and arsenate, the reduction and methylation of arsenic, and the biosynthesis of organoarsenic compounds represented in Figure 1.1.

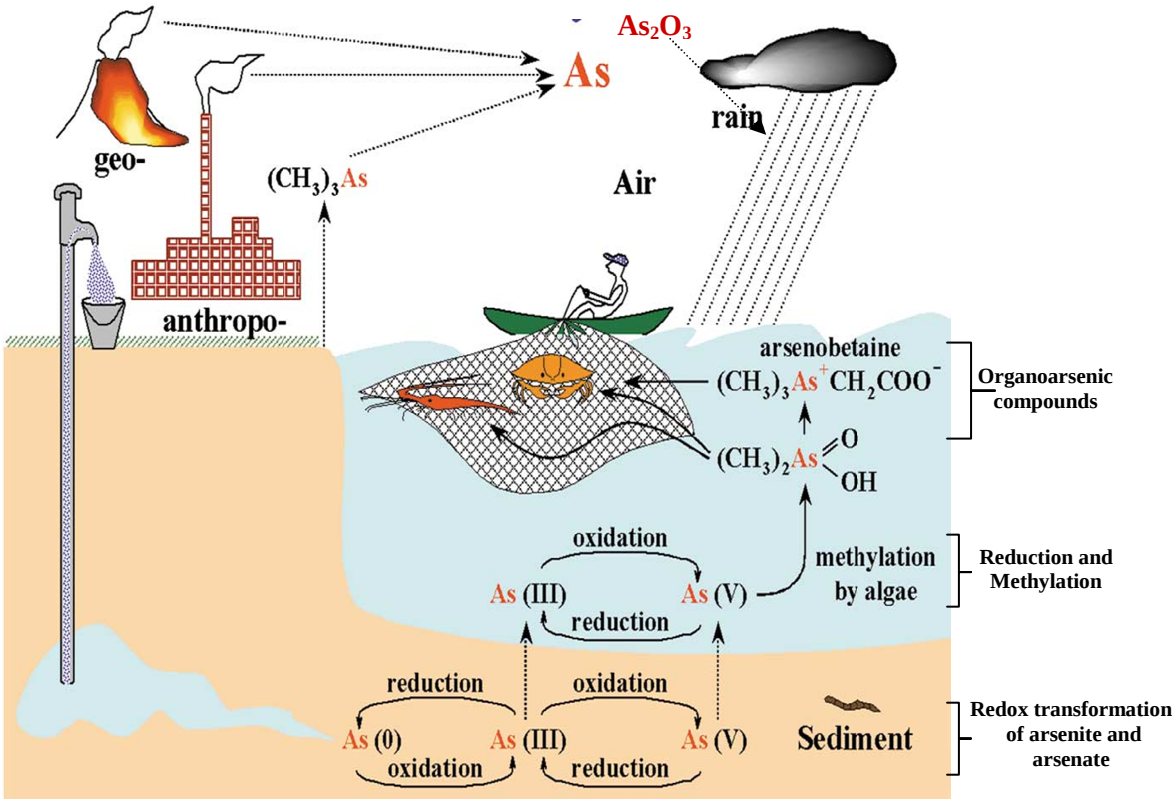

Figure 1.1 Bio-transformation of arsenic in environment through arsenic cycle
Source: Center for Computational Physics University of Coimbra.
Available at: http://www.viveraciencia.org/

1.5 Recommended value of arsenic in drinking water set by WHO

According to WHO, arsenic is one of 10 chemicals of major public health concern. The toxic effects of arsenic depend on the nature and extent of exposure, particularly the frequency of exposure, duration of exposure and type of arsenic present. A lot of efforts were given by WHO to reduce arsenic poisoning includes setting guideline values, reviewing evidence and providing risk management recommendations. WHO publishes a guideline value for arsenic in its Guidelines for Drinking-Water Quality. WHO established 200 µg/L as an allowable concentration in the first version of International Standards for Drinking-Water in 1958. In 1963, in the second version a stricter concentration of 50 µg/L arsenic was set as a new standard. In the last edition of the WHO Guidelines for Drinking-Water Quality published in 1993, a further stricter standard of 10 µg/L arsenic was suggested. In 2001, EPA in the United States adopted a reduced standard of 10 µg/L for public water supplies. However, highly contaminated countries of the world such as Bangladesh and India

have set up their standards 5 times higher than the maximum permissive limit set by WHO. Thus the maximum permissive limit of arsenic in drinking water set by Bangladesh Government is 50 µg/L. The intention of the guidelines is to be used as the basis for regulation and standard setting worldwide, for the development of national standards that, if properly implemented, will ensure the safety of drinking water supplies through the elimination, or reduction to a minimum concentration, of constituents in drinking water that are known to be hazardous to public health. The standard value of arsenic in guideline was designated as provisional due to the measurement difficulties and practical difficulties in removing arsenic from drinking water. It is when difficult to achieve the guideline value, member countries may set higher values as standards taking into account local circumstances, resources and risks.

1.6 Arsenic contamination in the world

Since ancient times, arsenic is considered as one of the most notorious poisons. Historical records show that arsenic was used by ancient Greeks, Persians, Romans and Chinese. Arsenic is used worldwide as an ingredient of a different kind of products in manufacturing industries, i.e. wood preservatives, herbicides, insecticides, pesticides, fungicides, high-emitting diodes, semi-conductors etc. resulting the work places become a source of inhalation and dermal exposure to arsenic for human population. On the other hand, groundwater in the different regions of the world is contaminated with arsenic. As a result, arsenic toxicity has created a major public health concern throughout the world. A major and possibly the dominant route of arsenic exposure to human and livestock is drinking of arsenic-contaminated groundwater. Millions of people are exposed to elevated levels of toxic inorganic arsenic through the contaminated drinking water and food (Meharg, 2008; Ng et al., 2003; Smith et al., 2000). Most severely affected countries that include Bangladesh, China, Nepal, India, Myanmar, Pakistan, Cambodia, Vietnam, Argentina, Australia, Canada, Greece, Chile, Hungary, Japan, Mexico, Mongolia, New Zealand, South Africa, Philippines, Taiwan, Thailand, USA, etc. where the main source of human exposure is the mass use of arsenic-contaminated groundwater for drinking and irrigation purposes (Berg et al., 2007; IARC, 2004; Nicolli et al., 1989; Nordstrom, 2002; Razo et al., 1990; Tseng, 1999).

In Asia, arsenic menace is more devastating in some countries specially the arsenic poisoning in alluvial deltas of Gangs River in India and Bangladesh (Bhattacharya et al., 1997; Dhar et al., 1997; Mandal et al., 1996; Nickson et al., 1998; Rahman et al., 2001; Smith et al., 2000). Groundwater of Pakistan, neighboring country of India and Bangladesh has been contaminated by arsenic above 10µg/L (Ahmad et al., 2004). Sun (2004) reported that the groundwater of the large areas of China and Inner Mongolia has also been contaminated with arsenic resulting in the toxicity of millions of people. Residents in the large alluvial deltas of the Mekong River in southern Vietnam and Cambodia and the Red River in northern Vietnam have been exposed to arsenic mainly through drinking water reported by Berg et al. (2007). Taiwan is another severely arsenic affected area in Asia. In the 1960s, Tseng et al. (1968, 1977) in Taiwan conducted epidemiologic studies and showed a strong association and dose-response relationship between high concentrations of arsenic in drinking water taken from the artesian wells and skin cancer, keratosis and blackfoot disease. Recently, groundwater contamination by arsenic has also been reported in Iran (Mosaferi et al., 2008).

In Europe, the arsenic problem is most alarming in Hungary, Serbia and Croatia (Gurzau and Gurzau, 2001; Sancha and Castro, 2001) where in Hungary an inventory of groundwater quality conducted which demonstrated that arsenic concentrations in drinking water for 400 towns and villages in the Great Hungarian Plain are several times higher than the guidelines of WHO and European Union (Csalagovits, 1999).

In the American region, Mexico, United States, Chile and Argentina are the most affected areas and some wells in Latin America including Bolivia and Peru, extremely high concentrations of arsenic were found (Nicolli et al., 1989; Razo et al., 1990; Welch et al., 1988). Different regions of the world having arsenic-contaminated groundwater are shown in Figure 1.2.

Figure 1.2 Arsenic in groundwater across the world
Available at: http://www.sickkids.ca/PGPR/Symposia-and-Workshops/Oct-2007-china/arsenic-pollution/index.html

1.7 Arsenic contamination: Bangladesh perspective

Bangladesh is considered a land of natural disasters because very often it has to face floods, cyclones, tidal bores, droughts etc. Bangladesh is an agro based country where surface and groundwater is extensively used for irrigation purposes. There is an abundance of surface and groundwater because of its flat deltaic land formed by the mechanical action of the great Himalayan rivers the Ganges, Brahmaputra and Meghna (Safiullah, 2006). Unfortunately, Bangladesh bears the scar of poisoning by the contamination of groundwater with arsenic. Here, the groundwater used for drinking has been contaminated with naturally occurring inorganic arsenic which poses a severe threat to the public health. The scale of this environmental disaster is

greater than any such event seen before. WHO described the arsenic crisis in Bangladesh as the largest mass poisoning of the twentieth century (Smith et al., 2000; WHO, 2000). Naturally-occurring arsenic contaminated groundwater was first detected in Bangladesh in 1993 by the Department of Public Health Engineering (DPHE) in the Nawabganj district. Bangladesh had to face a high rate infant mortality due to drinking of unsafe surface water from hand-dug wells, rivers and ponds which are contaminated by pathogenic bacteria. In the beginning of the 1970s, Department of Public Health Engineering, Government of Bangladesh, and United Nations Children's Fund (UNICEF) had set up millions of hand-pumped tube wells to get rid from this burden of water-borne diseases (Ahsan et al., 2000; Smith et al., 2000). Certainly, the incidence of the water-borne diseases was decreased. It was considered as a huge success for concern agencies since the primary goal of hand-pumped tube well was achieved. Unfortunately the situation became complex when in 1993, a substantial proportion of the tube wells yielding with high levels of soluble arsenic contaminated water were found (WHO, 2000). After then a series of surveys were conducted in between 1995-1998 which revealed that the groundwater of southern and north-eastern Bangladesh has been extensively contaminated with arsenic (BGS, 1999; Khan et al., 2003; Smith et al., 2000; Watanabe et al., 2001). In 1998, a British Geological Survey (BGS) collected more than 2000 water samples from 41 of the worst-affected districts. This project tested one tube well in every 37 Km^2 in the two third of the country's most affected areas and found that, 51% of the tube wells were contaminated with at least 10 μg/L, 35% with at least 50 μg/L, and rest of them were with at least 100 μg/L or more (BGS, 1999). Already an enormous number of toxicity cases have been reported in the North-West region of Bangladesh and approximately in between 80 to 100 millions of additional people are at risk for arsenic toxicity in the country (Caldwell et al., 2003; Chowdhury, 2004). The situation is deteriorating as the new cases of toxicity are still being reported in different parts of the country. There are 61 one out of 64 districts (administrative blocks) has been affected by arsenic in Bangladesh (Khan et al., 2006). The districts in Bangladesh having arsenic contaminated drinking water exceeding WHO guidelines are shown in Figure 1.3.

In 2002 and 2003, there were 4.7 million tube wells in Bangladesh have been screened for arsenic and among those 1.4 million tube wells were found to contain arsenic above the Government drinking water standard of 50 μg/L and painted red

with rest of them of green (Mukherjee et al., 2006). Almost one in five tube wells is not providing arsenic-safe drinking water. According to a recent survey (2009) of Bangladesh Bureau of Statistics (BBS) and UNICEF, 12.6 percent of households, equivalent to about 20 million people still drink water containing arsenic. Arsenic becomes a serious public health concern and threat for agricultural sustainability here in the country. Along with domestic use, huge amount of water from shallow aquifers is being used for irrigation of rice and other crops in the whole country. Most of the people in Bangladesh depend on groundwater as their primary source of drinking, cooking and irrigation which results in a significant amount of arsenic being cycled through environment each year with a sinister impact on public health and environment.

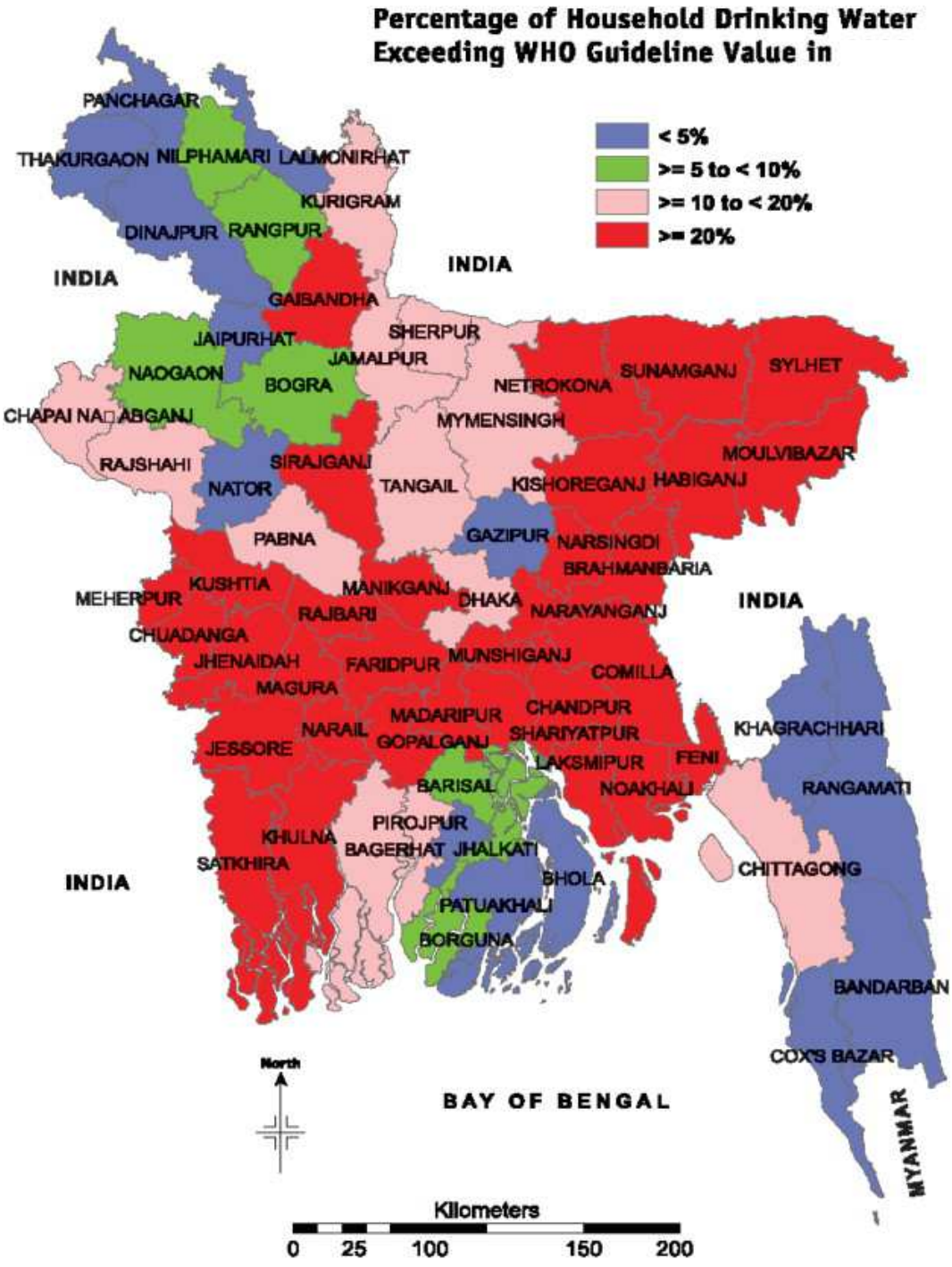

Figure 1.3 Arsenic polluted areas in Bangladesh. Arsenic contamination of household drinkingwater in Bangladesh in 2009 depicted by the Bangladesh Bureau of Statistics (BBS) and UNICEF Multiple Indicator Cluster Survey data.

1.8 Causes of arsenic contamination of groundwater in Bangladesh

There is a great temporal and spatial variation of groundwater arsenic level in different regions of Bangladesh. The precise reasons for the high level of arsenic in groundwater in Bangladesh is controversial issue and that have not yet been clearly established, although several theories have been proposed including role of microbial mobilization, anthropogenic activities, etc. (Harvey et al., 2002; Hossain et al., 2011; Islam et al., 2004; Polizzotto et al., 2006; Sutton et al., 2009). However, it is now widely believed that the elevated level of arsenic in groundwater in Bangladesh have a natural geological source which may be due to the abstraction of water from quaternary confined and semi-confined alluvial or deltaic aquifers. There are a huge number of diverse chemical and biological reactions such oxidation, reduction; adsorption, precipitation, methylation and volatilization participate actively in the cycling of arsenicals in groundwater. There are two principal theories of groundwater contamination by arsenic in Bangladesh.

1.8.1 Pyrite oxidation theory

Bangladesh is located in one of the major disaster and environmentally endangered areas of the world. In 1996, Das et al. conducted a geochemical survey in six districts of west Bengal, India bordering the western part of Bangladesh and the results indicate that the source of arsenic in groundwater and soil is the arsenopyrite. Arsenic pyrites or ferrous hydroxides are arsenic rich minerals which are generally stable in reducing environment under the water table and normally concentrated in organic deposits. However, it is not understood yet clearly about how arsenic is released in groundwater from arsenopyrite. In spite of being insoluble in water, pyrite decomposes when exposed to air or aerated water. A feasible explanation for this would be the changes of geochemical environment due to high withdrawal of groundwater resulting decomposition of pyrites to ferrous sulfate, ferric sulfate, and sulfuric acid, thus arsenic in pyrites becomes available (Bridge and Husain, 1999; Das et al., 1996; Welch et al., 1988). Due to massive withdrawal of undergroundwater in the last three decades, it may cause the decomposition of pyrites to oxides of iron, arsenic and sulphuric acid which are soluble in water containing sulphuric acid. In the reducing condition below the water table and in the presence of organic matter, non-toxic oxides of arsenic are reduced to toxic oxide forms.

1.8.2 Iron Oxyhydroxide reduction theory

In 1999, British Geological survey reported that the 'pyrite oxidation' hypothesis proposed by scientists from West Bengal is not a major process for arsenic recruitment in groundwater and also stated that ‘oxyhydroxide reduction’ hypothesis proposed by Nickson et al. (1998) is probably the main cause of arsenic mobilization in groundwater. According to this hypothesis, the origin of arsenic rich groundwater is due to a natural process, and it seems that the arsenic in groundwater has remained for thousands of years without being flushed from the delta. Arsenic is assumed to be present in alluvial sediments with high concentrations in sand grains as a coating of iron hydroxide. The sediments deposited in valleys eroded in the delta when the stream base level was lowered due to the drop in sea level during the last glacial advance. Organic matter deposited in the sediments reduce the arsenic adsorbed on the oxy-hydroxides and releases arsenic into the groundwater and dissolution occurs during recharge, caused by microbial oxidation of the organic matter as bacteria dissolves surrounding oxygen.

1.9 Metabolism of arsenic

The liver is the primary target organ for the metabolism of arsenic compound. The absorption of arsenic into the blood stream occurs at the cellular level and is taken up by red blood cells, white blood cells and other cells that can reduce arsenate to arsenite (Winski and Carter, 1995). Before methylation arsenate is reduced to arsenite form (Miller et al., 2002; Vahter, 2002; Vahter and Marafante, 1983). The primary metabolic step of inorganic arsenic in human is its methylation in the liver. The methylation of arsenic has been demonstrated by the presence of monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) in the urine and bile (Cui et al., 2004; Li et al., 2008). Arsenic (III) is methylated in the liver by arsenic methyltransferase (AS3MT) to generate monomethylarsonic acid (MMA) which is reduced to monomethylarsonous acid (MMA) and then further methylated to dimethylarsinic acid (DMA), followed by reduction to dimethylarsinous acid. In both step of methylation, S-adenosyl methionine provides methyl group. These metabolites are more readily excreted through urine. Some other less important routes of elimination of arsenic include feces, skin, sweat, hair and nails. Humans excrete a mixture of inorganic, monomethylated and dimethylated forms of arsenic. The

pentavalent metabolites MMA (V) and DMA (V) are less toxic than arsenite or arsenate (Marafante and Vahter, 1987). Inorganic arsenic and its methylated metabolites are mostly excreted through urine in 2–4 days. Approximately 50% of excreted arsenic in human urine is dimethylated and 25% is monomethylated, rest of them is inorganic (Buchet et al., 1981). Some studies related to arsenic metabolism have also suggested that methylation of inorganic arsenic may be a toxification, rather than a detoxification pathway since the trivalent methylated arsenic metabolites, particularly monomethylarsonous acid (MMA III) and dimethylarsinous acid (DMA III) are unusually capable of interacting with cellular targets such as proteins and DNA (Goering et al., 1999; Kitchin, 2001). Methylation capacity in human appears to decrease at high arsenic doses. The type and patterns of methylated arsenic species in urine are similar between siblings and between siblings and parents that indicates that arsenic methylation is genetically linked (Chung et al., 2002). When the methylating capacity of the liver is exceeded, exposure to excess levels of inorganic arsenic results in increased retention of arsenic in soft tissues.

1.10 Signs and symptoms of arsenicosis

Arsenicosis is a term defines as a chronic health condition arising from prolonged ingestion (not less than 6 months) of arsenic above a safe dose, usually manifested by characteristic skin lesions, with or without involvement of internal organs. Chronic exposure to increased levels of arsenic affects human health in many ways and manifests its signs and symptoms. Hallmark feature of chronic arsenic exposure include skin keratosis of the palms and soles, and hyper pigmentation of the limbs. Skin lesions usually develop after 5-10 years of exposure but shorter latencies are also possible. Long-term exposure to arsenic via contaminated drinking water can cause both acute and chronic toxic effects in all the organs and systems of the human body: skin, nervous system, liver, cardiovascular system, endocrine and respiratory system. Acute toxicity may occur in short duration but chronic arseicosis take longer period and may not become apparent clinically for a decade or more (Maidul et al., 1996). Severity of arsenic toxicity depends on chemical and physical form of arsenic compound, the route by which it enters the body, the dose and duration of exposure, dietary levels of interacting elements, age and sex of the individual (Khan et al., 1997). Symptoms of acute toxicity are severe projectile vomiting and watery diarrhea,

muscular cramp, facial edema, and cardiac abnormalities (Khan et. al., 1997; WHO, 1981). Chronic arsenic poisoning is associated with brown pigmentation and keratosis of palm, sole and rarely in the body along with other signs like anorexia, lethargy, diarrhoea or constipation, anemia, abdominal pain, neuropathy and so on (Bakshi, 1968; Hoque et al., 1996). Melanosis (spotted or diffuse), keratosis of palm and sole (spotted or diffuse), leukomelanosis (rain drop pigmentation), and hyperkeratosis are predominant skin symptoms of arsenicosis patients in Bangladesh. Melanosis is not always associated with keratosis but keratosis is always associated with melanosis.

1.11 Biomarkers of arsenic

A biomarker is defined as a xenobiotically induced alteration in cellular or biochemical components or processes, structures or functions, which is measurable in a biological system or sample. Biomarkers are classified as those of exposure, effect, and susceptibility. In toxicological point of view selection of appropriate biomarkers to be used for risk assessment is very much important and selection of biomarkers is based on the mechanism of a chemically induced disease state (Goyer, 1996). Arsenic is ubiquitously present in the environment. Contaminated drinking water and foods are the principal routes of human exposure to arsenic. After absorption arsenic rapidly distributed throughout the body via blood circulation and highest concentrations accumulated in skin, nails and hair. In epidemiology several biomarkers have been identified and used as arsenic exposure makers. Blood, urine, hair, nails are the most commonly used biological samples for different assays studying health risk of arsenic exposure.

Blood: Blood arsenic levels are best suited to evaluate the recent exposure to high dose of arsenic exposures. Speciation of blood arsenic has been used as a biomarker of arsenic exposure in many epidemiological studies using plasma, serum, and hemolyzed blood (Bemramdane et al., 1999; Ebdon et al., 1999; Huang et al., 2008; Mandal et al., 2004; Shibata et al., 1994; Slejkovec et al., 2008). Quantification of arsenic in the blood can be useful but it depends on the kind of epidemiological study. To study the dose–response relationship of individuals with ongoing chronic exposure and over a wide range of arsenic exposures are best by analyzing total blood arsenic concentrations (Hall et al., 2006; Mandal et al., 2004). Recent seafood ingestion by the subjects may show interference in the analysis of total arsenic in blood (Hughes,

2006). In addition, blood samples are more difficult to obtain for epidemiological research because the sampling is invasive. So blood arsenic is not an ideal exposure biomarker but it could be interesting to develop its use in the investigation of the early biological arsenic effects which precede overt disease (Marchiset-Ferlay et al., 2012).

Urine: Urinary arsenic has been used as a biomarker mostly in the epidemiological studies. Total arsenic in urine has often been used as an indicator of recent arsenic exposure because most of the arsenic species are excreted from the body through urine. Many epidemiological studies demonstrated the correlation between water arsenic and urinary arsenic concentrations (Calderon et al., 1999; Lindberg et al., 2006; Mandal et al., 2001, 2004; Meza et al., 2004; Sun et al., 2007). Total arsenic as well as arsenic species [inorganic arsenic (iAs), methylarsonic acid (MMAV) and dimethylarsinic acid (DMAV)] have been measured from human urine samples in several studies. Urinary arsenic species are better indicator for assessing the health risk of arsenic toxicity and methylation capacity of human body (Chen et al., 2005; Tseng, 2007; Vahter et al., 1999). But interpretation of urinary arsenic as a biomarker of arsenic exposure is difficult, because many factors are associated with arsenic metabolism (speciation) including age, sex, interindividual variability, capacity of methylation, smoking status, absorption of arsenobetaine rich-products such as seafood, dietary intake, folate deficiency, ethnicity and chronic exposure (Marchiset-Ferlay et al., 2012).

Hair and nails: Arsenic concentration in hairs or nails has been used historically in forensic investigations to confirm acute poisoning, since high concentrations of arsenic can be detected at the base of the nail or hair up to 10–14 days following death (Slotnick and Nriagu, 2006). Human hair and nails arsenic concentrations have been reported to provide integrated measures of arsenic exposure (Agahian et al., 1990; Ali et al., 2010; Karagas et al., 1996). Sulfhydryl groups present in the keratin that comprises hair and nails, arsenic is therefore drawn to these parts of the body more exclusively. Since these tissues grow slowly, so they can be used as indicators of long-term exposure. Background arsenic levels in hair are <1 μg/g (Hindmarsh, 2002) and in nails range from < 1.5 to 7.7 μg/g (Agahian et al., 1990; Hinwood et al., 2003). Many epidemiological studies demonstrated strong relationship of arsenic concentration in drinking water with arsenic concentration in hair and nails (Ali et al., 2010; Cavar et al., 2005; Chiou et al., 1997; Hinwood et al., 2003; Karagas et al.,

2000; Mandal et al., 2004). Arsenic in human nails has been often associated with concentrations of arsenic in drinking water, air, soil, dust and food (Slotnick and Nriagu, 2006).

Hair concentrations represent immediate exposure, as one centimeter of hair reflects approximately one month of exposure. On the other hand, nails capture historical exposure from several months to a year prior to sampling (Michaud et al., 2004). Therefore, blood and urine arsenic concentration have been considered to reflect only recent and acute exposures, whereas arsenic concentrations in hair and nails reflect long-term exposure (Marchiset-Ferlay et al., 2012; Polissar et al., 1990; Pomroy et al., 1980).

1.12 Health effects of arsenic exposure

Long-term exposure to arsenic is a major threat to the public health in the world. The characteristic health effects that result from ingestion of arsenic contaminated drinking water are slowly manifested, and the diagnosis is usually straightforward. Several studies have clearly indicated that the toxicity of arsenic depends on the exposure dose, frequency and duration, age, and sex, as well as on individual susceptibilities, genetic and nutritional factors (Ahsan et al., 2007; Maharjan et al., 2007; Milton et al., 2004; WHO, 2000). Chronic exposure to arsenic in drinking water is causally related to increased risks of cancer in the skin, lung, bladder, kidney, liver, colon, and prostate (Chen et al., 2003a, 2003b; Smith et al., 1998). Many epidemiological studies have also shown that arsenic exposure is associated with a number of non-neoplastic diseases including cardiac disease, cerebrovascular disease, pulmonary disease, peripheral neuropathy, diabetes mellitus and different diseases of the arteries, arterioles, and capillaries (Engel and Smith, 2004; Lee et al., 2002; Rahman et al., 1999; Tseng et al., 2003). In fact, arsenic affects almost all vital organs and systems of human body causing the damage or dysfunction (Hossain et al., 2012; Islam et al., 2011; Mandal et al., 2002). Adverse health effects of arsenic toxicity are manifested by increasing or decreasing levels of several soluble molecules and enzymes in human blood.

1.12.1 Dermatological effects

Skin lesions are a classical sign of chronic arsenic poisoning. Chronic exposure to arsenic by either ingestion or inhalation produce a variety of skin symptoms including

diffused and spotted melanosis, leucomelanosis, keratosis, hyperkeratosis, dorsum, Bowen's disease, and cancer. Skin disorders are well documented in several epidemiological studies conducted in different parts of the world in which the population are exposed to arsenic through drinking water (Ahsan et al., 2006; Chakraborti et al., 2003; Khan et al., 2003; Mazumder et al., 1998; Rahman et al., 2005). Resently, Yoshida et al. (2004) reported dose-response relationship between arsenic levels in drinking water and risk of skin lesions. Melanosis and keratosis are found at the primary stage of arsenic-induced all dermatological manifestations, leuko-melanosis and hyperkeratosis in the second stage and ultimately may turn to skin cancer such as Bowen's disease, basal cell and squamous cell carcinoma (Khan et al., 2003; Milton et al., 2003, Yoshida et al., 2004). Hyperpigmentation may occur, particularly in body areas where the skin tends to be a little darker (Shannon and Strayer, 1989). Photograph of some skin lesions are given below.

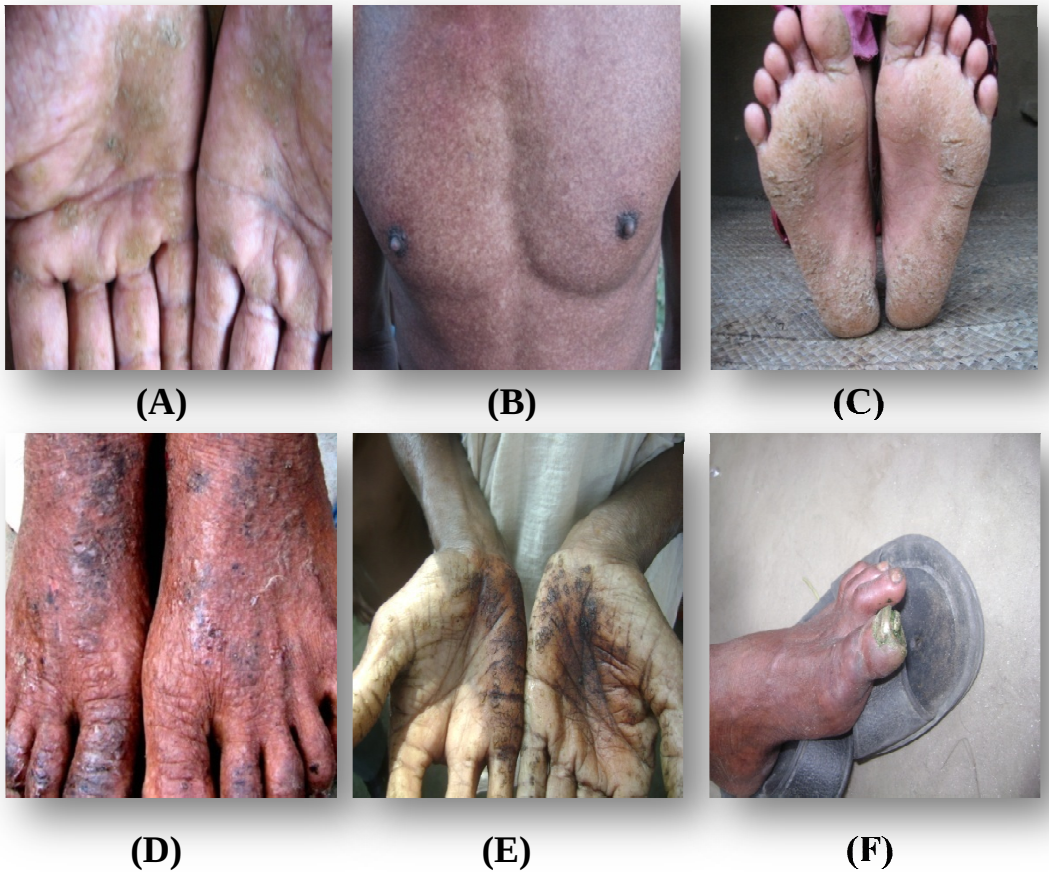

Figure 1.4 Some typical symptoms of arsenicosis. Diffuse or spotted melanosis on palms (A) and on chest (B), blister on the soles (C), hyperkeratosis and ulceration on foots (D) and on palm (E), gangrene in a big toe (F).

[**Source:** Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi, Bangladesh]

1.12.2 Carcinogenic effects

The International Agency for Research on Cancer (IARC) has classified inorganic arsenic as Group 1 human carcinogen (IARC, 1987). Many different systems or organs within the body are affected by chronic exposure to inorganic arsenic, particularly because of its potential as a human carcinogen. A carcinogen is any substance that directly involved in causing cancer. Cancer is the leading cause of arsenic-related mortality worldwide. The carcinogenic potential of arsenic was recognized over 110 years ago by Hutchison, who observed an unusual number of skin cancer in patients treated for various diseases with medical arsenicals (Klassen, 2008). Epidemiological studies have been carried out in different countries across the world which clearly demonstrated an evident causal relationship between environmental, occupational and medical exposure to inorganic arsenic and increased risks of cancer of the skin, lungs, urinary bladder, kidney, prostate, liver and other sites (Chen et al., 1992; IARC 2004; Smith et al., 1998; Wu et al., 1989; Yu et al., 2000). Ecological studies in Taiwan, Chile, Argentina and Australia and cohort studies from Taiwan and the USA demonstrated that long-term arsenic exposure increased the risks for kidney cancer (Chen et al., 1985, 1988a, 1992; Hopenhayn-Rich et al., 1998; Kurttio et al., 1999). Lung cancer is the leading cause of cancer-related mortality in the United States and also in rest of the world. An association between lung cancer and exposure to inorganic arsenic through different sources has been confirmed in several epidemiologic studies (Boyle and Maisonneuve, 1995; Hopenhayn-Rich et al., 1998). Liver is listed as a potential target organ for arsenic-induced carcinogenesis. Liver cancers can be developed from specific chronic liver diseases. Liver cirrhosis appears to be a primary cause of arsenic-related mortality in China, and is potentially associated with hepatocellular carcinoma (Liu et al., 1992, 2002). The association between environmental arsenic exposure and human liver cancers has been repeatedly reported (Centeno et al., 2002; Chiu et al., 2004; Liaw et al., 2008).

1.12.3 Cardiovascular effects

Long-term arsenic exposure is associated with increased morbidity and mortality from CVDs. CVDs are leading cause of death globally. Atherosclerosis is the fundamental step for the development of CVDs. Proatherogenic action of arsenic has been reported in several studies (Chen et al., 1988b; Chiou et al., 1997; Hossain et al.,

2012; Rahman et al., 1999; Sohel et al., 2009; Tseng et al., 2003, 2005; Wang et al., 2002). Cardiovascular system is a very sensitive target of arsenic toxicity. Arsenic-induced CVDs in human may result from the interaction among genetic, environment and nutritional factors. Epidemiological studies have shown that arsenic ingestion through food or water may have serious effects on the human cardiovascular system including heart damage (myocardial depolarization, hypertrophy of the ventricular wall, cardiac arrhythmias), vascular damage (Raynaud's disease, blackfoot disease, arterial thickening), ischemic heart disease, cerebrovascular diseases, and hypertension (Chen et al., 1988b; Chiou et al., 1997; Hossain et al., 2012; Rahman et al., 1999; Sohel et al., 2009; Tseng et al., 2003, 2005; Wang et al., 2002). The first evidence of a link between CVDs and arsenic in drinking water came in 1980 from Antofagasta, Chile, with a report of 17 deaths from myocardial infarction in people under the age of 40 (Yuan et al., 2007). Increased risk of CVDs was reported in smelter workers due to arsenic exposure (Axelson et al., 1978; Lee-Feldstein 1989). It is believed that vascular endothelial cells play a pivotal role in arsenic-induced cardiovascular diseases. Several epidemiological studies reported that chronic arsenic poisoning through ingestion of arsenic contaminated water can affects the platelets which increase the risk of death in humans from various form of CVDs (Axelson et al., 1978; Lee et al., 2002; Lee-Feldstein, 1983; Wu et al., 1989). A dose-response relationship between prevalence of CVD and ingested arsenic was reported in north-eastern Taiwan (Chiou et al., 1997). Ischemia is localized tissue anaemia due to obstruction of the inflow of arterial blood. Mounting evidence indicated that arsenic increases mortality from ischemic heart disease (Chang et al., 2004; Chen et al., 2011; Tsai et al., 1999). Black foot disease a unique form of peripheral vascular disease, has been reported to be one of the important complication of chronic arsenic toxicity in south-western Taiwan (Tseng, 1977). It is characterized by the severe systemic arteriosclerosis as well as dry gangrene and spontaneous amputations of affected extremities at end stages. Black foot disease has been mainly observed in Taiwan and it is possible that malnutrition contributes to its development. Increased prevalence of peripheral vascular disease has also been reported among residents with chronic arsenic exposure through drinking water in Taiwan, Chile, the USA, and Mexico (Chen et al., 2007; NRC, 1999, 2001; Tseng et al., 1996; Wang et al., 2007).

1.12.4 Respiratory effects

Chronic exposure to inorganic arsenic naturally and occupationally is associated with variety of respiratory problems including laryngitis, tracheae bronchitis, rhinitis, pharyngitis, shortness of breath, chest sounds, nasal congestion, hoarseness and chronic cough and perforation of the nasal septum (Dekundt et al., 1986; Mazumder et al., 2000; Milton and Rahman, 2002). The relationship between ingested arsenic and non-malignant respiratory effects has been reported from Chile, India, and Bangladesh (Smith et al., 1998). A fatal case of arsenic trioxide inhalation manifested widespread as tracheobronchial mucosal and sub mucosal haemorrhages with mucosal sloughing, alveolar haemorrhages, and pulmonary edema (Gerhardsson et al., 1988). Chronic asthmatic bronchitis and asthma is a common complication of groundwater arsenic toxicity (Saha, 1995).

1.12.5 Hepatotoxic effects

Liver is the primary target organ for arsenic metabolism. Since the liver tends to accumulate arsenic with repeated exposures, hepatic involvement is reported most commonly as a complication of chronic arsenic exposure. Abnormal liver function manifested by gastrointestinal symptoms such as abdominal pain, indigestion, loss of appetite and by clinical elevations of serum enzymes, frequently occurs from exposure to arsenic (Mazumder, 2005; Liu et al., 1992). Very recently Islam et al., (2011) have demonstrated the dose-response relationship between arsenic exposure and serum hepatic enzymes used for liver function test in the individuals exposed to arsenic in Bangladesh. Liver injury caused by chronic arsenic exposure is initially manifested by degenerative lesions with jaundice, progressing to non cirrhotic portal hypertension, fibrosis, cirrhosis and neoplasia such as hepacellular carcinoma (Centeno et al., 2002; Lu et al., 2001). Histological examination of the livers has revealed a consistent finding of portal tract fibrosis (Mazumder, 2005). The individuals who exposed more frequently to arsenic suffer from cirrhosis, which is considered to be a secondary effect of damage to the hepatic blood vessels. Hospitalized Indian arsenicosis patients have very high rates of hepatoportal sclerosis developed from drinking water contaminated with high level of arsenic (Dhawan et al., 1983; Santra et al., 1999). Chronic arsenic exposure in animals can also produce liver endothelial cell damage, which subsequently damages parenchymal cells (Straub

et al., 2007). All these studies clearly revealed that prolonged exposure to arsenic is associated with hepatomegaly, hepatic fibrosis and cirrhosis.

1.12.6 Renal effects

The kidneys are another important organ that accumulates inorganic arsenic with frequent exposure. Several epidemiological studies have shown the relationship between renal dysfunction and arsenic exposure through drinking water (Chen et al., 2011; Feng et al., 2013). The kidneys are the major route of arsenic excretion, as well as major site of conversion of pentavalent arsenic into the more toxic and less soluble trivalent arsenic (Tchounwou et al., 2003). Arsenic concentrates in the kidney during its urinary elimination affects the function of proximal convoluted tubules (Burton et al., 1995; Parrish et al., 1999). Acute renal dysfunction due to arsenic exposure is characterized by acute tubular necrosis and cast formation with increase in blood urea nitrogen and creatinine levels (Kimura et al., 2006). Several animal studies have reported renal effects following intermediate or chronic-duration of oral arsenic exposure (Brown et al., 1976, Karim et al., 2010, Rahman et al., 2012). The effects caused by arsenic include increased kidney weight, inflamed mitochondria and increased numbers of dense autophagic lysosome-like bodies in the proximal tubules, increased pigmentation in the proximal tubules, and cysts.

1.12.7 Neurological effects

Exposure to arsenic is associated with several form of neurological complications including impaired memory, poor concentration, Parkinson's disease, Guillain-Barre like neuropathy, verbal comprehension, encephalopathy, and peripheral neuropathy (Bardullas et al., 2009; Piao et al., 2005; Vahidnia et al., 2006; Yip et al., 2002). The postulated mechanism for arsenic-induced neurotoxicity majorly involves oxidative stress with increased reactive oxygen species, lipid peroxides along with decrease in superoxide dismutase and reduced glutathione levels (Dwivedi and Flora, 2011). Arsenic exposure has been reported to alter metabolism of various neurotransmitters such as monoamines, acetylcholine, gamma amino butyric acid and glutamate (Rodriguez et al., 2002). The deficiency of thiamine is well known to induce neuronal complications. Arsenic exposure causes thiamine deficiency and inhibits pyruvate decarboxylase which elevates blood pyruvate and hence causes encephalopathy (Gopalkrisnan and Rao, 2006). Symptoms of chronic encephalopathy

include persistent headache, diminished recent memory, distractibility, abnormal irritability, restless sleep, loss of libido, increased urinary urgency, and a bit increased effects of ethanol (Morton and Caron, 1989). Mental health problem (depression) is common neurological problem in the arsenic-endemic people in Bangladesh and China (Brinkel et al., 2009). A significant association between decreased reading and spelling performance and hair arsenic levels was found in a group of elementary school children suggesting that arsenic may also cause neurobehavioral effects (Moon et al., 1985).

1.12.8 Reproductive effects

Arsenic is a reproductive toxicant and a teratogen (Shalat, 1996). Arsenic and its methylated metabolites are reported to cross the placenta in both human and animals and thus arsenic is easily transferred to fetus at least in late stage of gestation (Concha et al., 1998; He et al., 2007). Several studies suggest that there is an association between arsenic exposure and adverse pregnancy outcomes, such as spontaneous abortion, stillbirth, low birth weights, fetal loss and infant death (Ahmad et al., 2001; Milton et al., 2005; Rahman et al., 2007; von Ehrenstein et al., 2006). A significant decrease in sperm count and motility along with increase in abnormal sperm were observed at high concentration of arsenic exposure in mice (Pant et al., 2001). Arsenic exposure also affects weight and length during infancy and early childhood (Saha et al., 2012).

1.12.9 Genotoxic effects

Inorganic arsenic is generally recognized as a clastogenic agent. Several studies have been carried out exploring the genotoxic effect of inorganic arsenicals (Cohen et al., 2006; Yamanaka et al., 2004). There are divers mechanisms of arsenic-induced genotoxic damages, chromosomal abnormalities, epigenetic changes that alter DNA methylation have been proposed (Chanda et al., 2006; Kitchin, 2001; Rossman et al., 2004; Zhao et al., 1997). Chromosomal aberrations, DNA-protein cross-links, and sister chromatid exchanges were observed in hamster embryo cells, human lymphocytes and fibroblasts after exposure to inorganic arsenic (Dong and Luo, 1993; Jha et al., 1992; Kochhar et al., 1996; Lee et al., 1985; Okui and Fujiwara, 1986; Rasmussen and Menzel, 1997; Wiencke and Yager, 1991). Arsenic-induced chromosomal aberrations are characterized by chromatid gaps, and fragmentation,

endoreduplication, and chromosomal breaks. It has already been reported that both arsenite and its metabolites can have a variety of genotoxic effects, which may be mediated by oxidants or free radical species. All of these species also have effects on signaling pathways leading to proliferative responses. There are interesting differences in the activities of inorganic and organic species both in terms of target organ carcinogenicity, toxic and genotoxic mechanisms. Mass et al. (2001) indicated that exposure of human lymphocytes to methylated trivalent arsenic causes direct DNA damage. A study using an earlier version of the alkaline elution method has indicated that arsenic induces DNA strand breaks in human fatal lung fibroblasts (Dong and Luo, 1993). Vuyyuri et al. (2006) reported that occupational exposure to arsenic among workers in a glass plant in India whose levels of blood arsenic were five times higher than in the control group had increased DNA damage in leukocytes. Li et al. (2001) reported that arsenic induced typical and various extents of DNA strand breaks in human cells via reactive oxygen species (ROS) in a dose-dependent manner. The most extensively studied DNA lesion is the formation of 8-hydroxyguanine (8-OH-G), one of the major products of DNA oxidation, which originates from the reaction of hydroxyl radical with guanine (Valko et al., 2006). 8-OH-G is a sensitive genotoxic marker of oxidative damaged DNA. Associations of arsenic exposure with increased urinary 8-OH-G concentrations have also been observed (Hu et al., 2006).

1.13 Dissertation aim

Arsenic is a potent environmental pollutant and well established human carcinogen. Arsenic is widely present in water, food, soil, and airborne particles. It is released in the environment from both natural and man-made sources. Ingestion of inorganic arsenic have been documented to be associated with a variety of diseases including cancers, CVDs, dermatitis, neurotoxcity, diabetes mellitus, renal failure and liver dysfunction (Chen and Ahsan, 2004; Hossain et al., 2012; Islam et al., 2011; Mazumder et al., 1998; Meliker et al., 2007; Tapio and Grosche, 2006; Vahidnia et al., 2008; Wang et al., 2002). CVDs and cancer are the leading causes of arsenic-related morbidity and mortality globally (Smith et al., 2000; Chen et al., 1988b; Sohel et al., 2009; Lokuge et al., 2004). CVDs refer to diseases that affect the heart or the blood vessel system within a person's entire body and it is often manifest clinically as

hypertension, coronary disease, stroke or peripheral arterial diseases (Scott et al., 1999). Atherosclerosis is the fundamental step for the development of CVDs. Cardiovascular system is a very sensitive target of arsenic toxicity. Proatherogenic action of arsenic has been reported in several studies (Chen et al., 1988b; Chiou et al., 1997; Hossain et al., 2012; Rahman et al., 1999; Sohel et al., 2009; Tseng et al., 2003; Wang et al., 2002). In fact, atherosclerosis is a multi-factorial disease mainly caused by hardening of the arteries due to a thickening of the artery lining from fatty deposits or plaques. Chronic inflammation has an established role in the pathogenesis and progression of atherosclerosis (Libby, 2002; Ross, 1999; Steinberg, 2002). There are several inflammatory and cell adhesion molecules such as C-reactive protein (CRP), intercellular adhesion molecule-1(ICAM-1), vascular cell adhesion molecule (VCAM-1) related to the biochemical event of atherosclerosis (Paffen et al., 2006; Ross, 1999).

Angiogenesis is the process that new capillaries are generated from the pre-existing vasculature which plays a pivotal role in the initiation of carcinogenesis and tumor progress, vascular diseases, and various ischemic and inflammatory diseases (Carmeliet and Jain, 2000; Folkman, 1971). Vascular endothelial growth factor (VEGF) is a potent angiogenic factor whose activities include endothelial cell survival, proliferation, migration, and tube formation. Tumors are not able to grow beyond a limited size (1~2 mm) without adequate blood supply. Because of its predominating role in angiogenesis compared to the other molecules, VEGF has been recognized as a biomarker for angiogenesis (Murukesh et at., 2010). Endothelial cells are the major target of arsenic-related CVDs and cancer but significant gaps remain in the mechanistic understanding of arsenic-induced endothelial cell dysfunction and pathogenesis. This study has been designed to explore the association of arsenic exposure with the circulating inflammatory, adhesion and angiogenic molecules implicated in CVDs and cancer (Chapter 2 and 3).

1.14 References

Agahian, B., Lee, J. S., Nelson, J. H., and Johns, R. E. (1990). Arsenic levels in fingernails as a biological indicator of exposure to arsenic. *Am. Ind. Hyg. Assoc. J.* **51**, 646-651.

Ali, N., Hoque, M. A., Haque, A., Salam, K. A., Karim, M. R., Rahman, A., Islam, K., Saud, Z. A., Khalek, M. A., Akhand, A. A., Hossain, M., Mandal, A., Karim, M. R., Miyataka, H., Himeno, S., and Hossain, K. (2010). Association between arsenics exposure and plasma cholinesterase activity: a population based study in Bangladesh. *Environ. Health* **9**, 36.

Ahmad, S. A., Sayed, M. H. S. U., Barua, S., Khan, M. H., Faruquee, M. H., Jalil, A., Hadi, S. A., and Talukder, H. K. (2001). Arsenic in drinking water and pregnancy outcomes. *Environ. Health Perspect.* **109**, 629-631.

Ahmad, T., Kahlown, M. A., Tahir, A., and Rashid, H. (2004). Arsenic an emerging issue: experience from Pakistan. People-centered approaches to water and environ-mental sanitation. In: Thirtieth WEDC International Conference, 25–29 October 2004,Vientiane, LaoPDR,WEDC, pp.459-466.

Ahsan, H., Chen, Y., Kibriya, M. G., Slavkovich, V., Parvez, F., Jasmine, F., Gamble, M. V., and Graziano, J. H. (2007). Arsenic metabolism, genetic susceptibility, and risk of premalignant skin lesions in Bangladesh. *Cancer. Epidemiol. Biomarkers Prev.***16,** 1270-1278.

Ahsan, H., Chen, Y., Parvez, F., Zablotska, L., Argos, M., Hussain, I., Momotaj, H., Levy, D., Cheng, Z., Slavkovich, V., van Geen, A., Howe, G. R., and Graziano, J. H. (2006). Arsenic exposure from drinking water and risk of premalignant skin lesions in Bangladesh: baseline results from the Health Effects of Arsenic Longitudinal Study. *Am. J. Epidemiol.* **163**, 1138-1148.

Ahsan, H., Perrin, M., Rahman, A., Parvez, F., Stute, M., Zheng, Y., Milton, A. H., Brandt-Rauf, P., van Geen, A., and Graziano, J. (2000). Associations between drinking water and urinary arsenic levels and skin lesions in Bangladesh. *J. Occup. Environ. Med.* **42,** 1195-1201.

Argos, M., Kalra, T., Rathouz, P. J., Chen, Y., Pierce, B., Parvez, F., Islam, T., Ahmed, A., Rakibuz-Zaman, M., Hasan, R., Sarwar, G., Slavkovich, V., van

Geen, A., Graziano, J., and Ahsan, H. (2010). Arsenic exposure from drinking water, and all-cause and chronic-disease mortalities in Bangladesh (HEALS): a prospective cohort study. *Lancet* **376**, 252-258.

Axelson, O., Dahlgren, E., Jansson, C. D., and Rehnlund, S. O. (1978). Arsenic exposure and mortality: A case reference study from a Swedish copper smelter. *Br. J. Ind. Med.* **35,** 8-15.

Bakshi, H. N. (1968). Synopsis of medical jurisprudence. 7th ed. Calcutta: Temple Press, 1968, 347-348.

Bardullas, U., Limón-Pacheco, J. H., Giordano, M., Carrizales, L., Mendoza-Trejo, M. S., and Rodríguez, V. M. (2009). Chronic low-level arsenic exposure causes gender-specific alterations in locomotor activity, dopaminergic systems, and thioredoxin expression in mice. *Toxicol. Appl. Pharmacol.* **239,** 169-177.

BBS & UNICE. (2009). Multiple Indicator Cluster Survey. Available at: http://www.unicef.org/bangladesh/knowledgecentre_6292.htm

Bemramdane, L., Accominotti, M., Fanton, L., Malicier, D., Vallon, J. J. (1999). Arsenic speciation in human organs following fatal arsenic trioxide poisoning—a case report. *Clin. Chem.* **45**, 301-306.

Berg, M., Stengel, C., Pham, T. K., Pham, H. V., Sampson, M. L., Leng, M., Samreth, S., and Fredericks, D. (2007). Magnitude of arsenic pollution in the Mekong and Red River Deltas–Cambodia and Vietnam. *Sci. Total. Environ.* **372**, 413-425.

BGS. (1999). Arsenic contamination of groundwater in Bangladesh: A review. 1999, S5:54. Available at: ttp://www.bgs.ac.uk/arsenic/

Bhattacharya, P., Chatterjee, D., and Jacks, G. (1997). Occurrence of arsenic-contaminated groundwater in alluvial aquifers from Delta plains, Eastern India: Options for safe drinking water supply. *Int. J. Water Resour. D.* **13**, 79-92.

Boyle, P., and Maisonneuve, P. (1995). Lung cancer and tobacco smoking. *Lung Cancer* **12**, 167–181.

Brammer, H. (2009). Mitigation of arsenic contamination in irrigated paddy soils in South and South-east Asia. *Environ. Int.* **35**, 856–863.

Brammer, H., and Ravenscroft, P. (2009). Arsenic in groundwater: A threat to sustainable agriculture in South and South-east Asia. *Environ. Int.* **35**, 647-654.

Bridge T, and Husain M. (1999). Ground water arsenic poisoning and a solution to arsenic disaster in Bangladesh. Arsenic International Conference, NY. The Daily Star (Bangladesh), News From Bangladesh (Bangladesh), The Weekly Bangla Barta, LA, USA, The Weekly Bangladesh, NY, USA and Internets.

Brinkel, J., Khan, M. M. H., and Kraemer, A. (2009). A systematic review of arsenic exposure and its social and mental health effects with special reference to Bangladesh. *Int. J. Environ. Res. Public. Health* **6,** 1609–1619.

Brown. M. M., Rhyne, B. C., Goyer, R. A., and Fowler, B. A. (1976). Intracellular effects of chronic arsenic administration on renal proximal tubule cells. *J. Toxicol. Environ. Health* **1,** 505–514.

Buchet, J. P., Lauwerys, R., and Roels, H. (1981). Comparison of the urinary excretion of arsenic metabolites after a single dose of sodium arsenite, monomethylarsonate or dimethylarsinate in man. *Int. Arch. Occup. Environ. Health* **48,** 71-179.

Buchet, J. P., Pauwels, J., and Lauwerys, R. (1984). Assessment of exposure to inorganic arsenic following ingestion of marine organisms by volunteers. *Environ. Res.* **66,** 44–51.

Burton, C. A., Hatlelid, K., Divine, K., Carter, D. E., Fernando, Q., Brendel, K., and Gandolfi, A. J. (1995). Glutathione effects on toxicity and uptake of mercuric chloride and sodium arsenite in rabbit renal cortical slices. *Environ. Health Perspect*. **103**, 81-84.

Calderon, R. L., Hudgens, E., Le, X. C., Schreinemachers, D., and Thomas, D. J. (1999). Excretion of Arsenic as a function of exposure to Arsenic in drinking water. *Environ. Health Perspect.***107**, 663–667.

Caldwell, B. K., Caldwell, J. C., Mitra, S. N., and Smith, W. (2003). Searching for an optimum solution to the Bangladesh arsenic crisis. *Soc. Sci. Med.* **56,** 2089-2096.

Carmeliet, P., and Jain, R. K. (2000). Angiogenesis in cancer and other diseases. *Nature* **407**, 249–257.

Cavar, S., Klapec, T., Grubesic, R. J., and Valek, M. (2005). High exposure to arsenic from drinking water at several localities in eastern Croatia. *Sci. Total. Environ.* **339**, 277–282.

Centeno, J. A., Mullick, F. G., Martinez, L., Page, N. P., Gibb, H., Longfellow, D., Thompson, C., and Ladich, E. R. (2002). Pathology related to chronic arsenic exposure. *Environ. Health Perspect*. **110**, 883–886.

Chakraborti, D., Hussam, A., and Alauddin, M., (2003). Arsenic: environmental and health aspects with special reference to groundwater in South Asia. Foreword. *J. Environ. Sci. Health A. Tox. Hazard Subst. Environ. Eng.* **38**, xi-xv.

Chanda, S., Dasgupta, U. B., Guhamazumder, D., Gupta, M., Chaudhuri, U., Lahiri, S., Das, S., Ghosh, N., and Chatterjee, D. (2006). DNA hypermethylation of promoter of gene p53 and p16 in arsenic-exposed people with and without malignancy. *Toxicol. Sci*. **89**, 431-437.

Chang, C. C., Ho, S. C., Tsai, S. S., and Yang, C. Y. (2004). Ischemic heart disease mortality reduction in an arseniasis-endemic area in southwestern Taiwan after a switch in the tap-water supply system. *J. Toxicol. Environ. Health A.* **67**, 1353-1361.

Chen, C. J., Chen, C. W., Wu, M. M., and Kuo, T. L. (1992). Cancer potential in liver, lung, bladder, and kidney due to ingested inorganic arsenic in drinking water. *Br. J. Cancer* **66,** 888-892.

Chen, C. J., Chuang, Y. C., Lin, T. M., and Wu, H. Y. (1985). Malignant neoplasms among residents of a Blackfoot disease-endemic area in Taiwan: High-arsenic artesian well water and cancers. *Cancer Res*. **45**, 5895-5899.

Chen, C. J., Hsu, L. I., Wang, C. H., Shih, W. L., Hsu, Y. H., Tseng, M. P., Lin, Y. C., Chou, W. L., Chen, C. Y., Lee, C. Y., Wang, L. H., Cheng, Y. C., Chen, C. L., Chen, S. Y., Wang, Y. H., Hsueh, Y. M., Chiou, H, Y., and Wu, M. M. (2005). Biomarkers of exposure, effect, and susceptibility of arsenic-induced health hazards in Taiwan. *Toxicol. Appl. Pharmacol*. **206**, 198-206.

Chen, C. J., Kuo, T. L., and Wu. M. M. (1988a). Arsenic and cancers. *Lancet* **1**, 414-415.

Chen, C. J., Wang, S. L., Chiou, J. M., Tseng, C. H., Chiou, H. Y., Hsueh, Y. M., Chen, S. Y., Wu, M. M., and Lai, M. S. (2007). Arsenic and diabetes and hypertension in human populations. *Toxicol. Appl. Pharmacol.* **222,** 298-304.

Chen, C. J., Wu, M. M., Lee, S. S., Wang, J. D., Cheng, S. H., and Wu, H. Y. (1988b). Atherogenicity and carcinogenicity of high-arsenic artesian well water. Multiple risk factors and related malignant neoplasms of blackfoot disease. *Arteriosclerosis* **8**, 452-460.

Chen, J. W., Chen, H. Y., Li, W. F., Liou, S. H., Chen, C. J., Wu, J. H., and Wang, S. L. (2011). The association between total urinary arsenic concentration and renal dysfunction in a community-based population from central Taiwan. *Chemosphere* **84,** 17-24.

Chen, Y., and Ahsan, H. (2004). Cancer burden from arsenic in drinking water in Bangladesh. *Am. J. Public Health* **94**, 741-744.

Chen, Y., Graziano, J. H., Parvez, F., Liu, M., Slavkovich, V., Kalra, T., Argos, M., Islam, T., Ahmed, A., Rakibuz-Zaman, M., Hasan, R., Sarwar, G., Levy, D., van Geen, A., and Ahsan, H. (2011). Arsenic exposure from drinking water and mortality from cardiovascular disease in Bangladesh: prospective cohort study. *BMJ* **362**, d2431.

Chen, Y. C., Guo, Y. L., Su, H. J., Hsueh, Y. M., Smith, T. J., Ryan, L. M., Lee, M. S., Chao, S. C., Lee, J. Y., and Christiani, D. C. (2003a). Arsenic methylation and skin cancer risk in southwestern Taiwan. *J. Occup. Environ. Med.* **45,** 241-248.

Chen, Y. C., Su, H. J., Guo, Y. L., Hsueh, Y. M., Smith, T. J., Ryan, L. M., Lee, M. S., Christiani, D. C. (2003b). Arsenic methylation and bladder cancer risk in Taiwan. *Cancer Cause. Control* **14**, 303–310.

Chiou, H. Y., Huang, W. I., Su, C. L., Chang, S. F., Hsu, Y. H., and Chen, C. J. (1997). Dose–response relationship between prevalence of cerebrovascular disease and ingested inorganic arsenic. *Stroke* **28**, 1717–1723.

Chowdhury, A. M. R. (2004). Arsenic crisis in Bangladesh. *Sci. Am.* **291**: 86-91.

Chowdhury, U. K., Biswas, B. K., Chowdhury, T. R., Samanta, G., Mandal, B. K., Basu, G. C., Chanda, C. R., Lodh, D., Saha, K. C., Mukherjee, S. K., Roy, S.,

Kabir, S., Quamruzzaman, Q., and Chakraborti, D. (2000). Groundwater arsenic contamination in Bangladesh and West Bengal, India. *Environ. Health Perspect.* **108,** 393-397.

Chung, J. S., Kalman, D. A., Moore, L. E., Kosnett, M. J., Arroyo, A. P., Beeris, M., Mazumder, D. N., Hernandez, A. L., and Smith, A. H. (2002). Family correlations of arsenic methylation patterns in children and parents exposed to high concentrations of arsenic in drinking water. *Environ. Health Perspect.* **110,** 729-733.

Cohen, S. M., Arnold, L. L., Eldan, M., Lewis, A. S., Beck, B. D. (2006). Methylated arsenicals: the implications of metabolism and carcinogenicity studies in rodents to human risk assessment. *Crit. Rev. Toxicol.* **36**, 99-133.

Colbourn, P., Alloway, B. J., and Thornton, I. (1975). Arsenic and heavy metals in soils associated with regional geochemical anomalies in S.W. England. *Sci.Total. Environ.* **4**, 359-363.

Concha, G., Vogler, G., Lezeano, D., Nermell, B., and Vahter, M. (1998). Exposure to inorganic arsenic metabolites during early human development. *Toxicol. Sci.* **44,** 185-190.

Csalagovits, I. (1999). Arsenic-bearing artesian waters of Hungary. In: Annual report of the Geological Institute of Hungary 1992-1993/II: 85-92.

Cui, X., Kobayashi, Y., Hayakawa, T., and Hirano, S. (2004). Arsenic speciation in bile and urine following oral and intravenous exposure to inorganic and organic arsenics in rats. *Toxicol. Sci*. **82,** 478-487.

Das, D., Samanta, G., Mandal, B. K., Chowdhury, T. R., Chanda, C. R., Chowdhury, P. P., Basu, G. K., and Chakraborti, D. (1996). Arsenic in groundwater in six districts of West Bengal, India. *Environ. Geochem. Health* **18,** 5-15.

Deknudt, G., Léonard, A., Arany, J., Jenar-Du Buisson, G., and Delavignette, E. (1986). In vivo studies in male mice on the mutagenesis effects of inorganic arsenic. *Mutagenesis* **1**, 33-34.

DG Environment, Ambient air pollution by As, Cd and Ni compounds, Position Paper, Working Group on Arsenic, Cadmium and Nickel Compounds, DG Environment European Commission, 2000.

Dhar, R. K., Biswas, B. K, Samanta, G., Mandal B. K., Chakraborti, D., Roy, S., Jafar, A., Islam, A., Ara, G., Kabir, S., Khan, A. W., Ahmed, S. A., and Hadi, S. A. (1997). Groundwater arsenic calamity in Bangladesh. *Curr. Sci.* **73**: 48-59.

Dhawan, D., Narang, A. P. S., and Datta, D. V. (1983). Levels of arsenic in liver cirrhosis. *Toxicol. Let.* **15**, 105-108.

Dong, J. T., and Luo, X. M. (1993). Arsenic-induced DNA-strand breaks associated with DNA-protein crosslinks in human fetal lung fibroblasts. *Mutat. Res.* **302**, 97-105.

Duxbury, J. M., Mayer, A. B., Lauren, J. G., and Hassan, N. (2003). Food chain aspects of arsenic contamination in Bangladesh: effects on quality and productivity of rice. *J. Environ. Sci. Health. A.* **38**, 61-69.

Dwivedi, N., and Flora, S. J. (2011). Concomitant exposure to arsenic and organophosphates on tissue oxidative stress in rats. *Food Chem. Toxicol*. **49,** 1152-1159.

Ebdon, L., Fisher, A., Roberts, N., and Yaqoob, M. (1999). Determination of organoarsenic species in blood plasma by HPLC-ICP MS. *Appl. Organometal. Chem*. **13,** 183-187.

Engel, R. R., and Smith, A. H., (2004). Arsenic in drinking water and mortality from vascular disease: an ecologic analysis in 30 countries in the United States. *Arch. Environ. Health* **49,** 418-427.

EPA. (1975). Interim primary drinking water standards. *Fed. Reg*. **40** (11), 990-11998.

Feng, H., Gao, Y., Zhao, L., Wei, Y., Li, Y., Wei, W., Wu, Y., and Sun, D. (2013). Biomarkers of renal toxicity caused by exposure to arsenic in drinking water. *Environ. Toxicol. Pharmacol*. **35**, 495-501.

Ferguson, J. F., and Gavis, J. (1972). A review of the arsenic cycle in natural waters. *Water Res.* **6**, 1259–1274.

Folkman, J. (1971). Tumor angiogenesis: therapeutic implications. *N. Engl. J. Med.* **285**, 1182-1186.

Francesconi, K. A., and Edmonds, J. S. (1997). Arsenic and marine organisms. *Adv. Inorg. Chem*. **44**, 147-189.

Garelick, H., Jones, H., Dybowska, A., and Valsami-Jones, E. (2008). Arsenic pollution sources. *Rev. Environ. Contam. Toxicol.* **197**, 17-60.

Gerhardsson, L., Dahlgren, E., Eriksson, A., Lagerkvist, B. E. A., Lundstrom, J., and Nordberg, G. P. (1988). Fatal arsenic poisoning-a case report. *Scand. J. Work Environ. Health* **14**, 130-133.

Goering, P. L., Aposhian, H. V., Mass, M. J., Cebrián, M., Beck, B. D., and Waalkes, M. P. (1999). The enigma of arsenic carcinogenesis: role of metabolism. *Toxicol. Sci*. **49,** 5-14.

Goldschmidt, V. M. (1954). Arsenic, in: A. Muir (Ed.), Geochemistry, Clarendon Press, Oxford, pp. 468-478.

Gopalkrishnan, A., and Rao, M. V. (2006). Amelioration by vitamin A upon arsenic induced metabolic and neurotoxic effects. *J. Health Sci*. **52**, 568-577.

Goyer, R. A. (1996). Toxic effects of metals. In: Casarett I, Louis J, Klaassen C, Amdur M, Doull S, editors. Casarett and Doull's toxicology: the basic science of the poisons. 5th ed. New-York: Mc Graw-Hill. 691-736.

Grotti, M., Soggia, F., Lagomarsino, C., Goessler, W., and Francesconi, K. A., (2008). Arsenobetaine is a significant arsenical constituent of the red Antarctic alga Phyllophora antarctica. *Environ. Chem.* **5,** 171-175.

Gurzau, E. S., and Gurzau, A. E. (2001). In: Cambell WR, Abernathy CO, Calderon RL, editors. Arsenic: exposure and health effects IV. Amsterdam: Elsevier, 81-184.

Hall, M., Chen, Y., Ahsan, H., Slavkovich, V., van Geen, A., Parvez, F., Graziano, J. (2006). Blood arsenic as a biomarker of arsenic exposure: results from a prospective study. *Toxicology* **225**, 225-233.

Harvey, C. F., Swartz, C. H., Badruzzaman, A. B. M., Keon-Blute, N., Yu, W., Ali, M. A., Jay, J., Beckie, R., Niedan, V., Brabander, D., Oates, P. M., Ashfaque, K.

N., Islam, S., Hemond, H. F, and Ahmad, M. F. (2002). Arsenic mobility and groundwater extraction in Bangladesh. *Science* **298**, 602-1606.

He, W., Greenwell, R. J., Brooks, D. M., Calderón-Garcidueñas, L., Beall, H. D., and Coffin, J. D. (2007). Arsenic exposure in pregnant mice disrupts placental vasculogenesis and causes spontaneous abortion. *Toxicol. Sci.* **99,** 244-253.

Hindmarsh, J. T. (2002). Caveats in hair analysis in chronic arsenic poisoning. *Clin. Biochem.* **35,** 1-11.

Hinwood, A. L., Sim, M. R., Jolley, D., de Klerk, N., Bastone, E. B., Gerostamoulos, J., and Drummer, O. H. (2003). Hair and toenail arsenic concentrations of residents living in areas with high environmental arsenic concentrations. *Environ. Health Perspect.* **111,** 187-193.

Hopenhayn-Rich, C., Biggs, M. L., and Smith, A. H. (1998). Lung and kidney cancer mortality associated with arsenic in drinking water in Cordoba, Argentina. *Int. J. Epidemiol.* **27,** 561-569.

Hoque, M. M., Amin, A. A., and Alam, M. M., (1996). Chronic arsenic poisoning in a village of Chapai nawabgonj district - A cross sectional study. TAJ, 9, 39-41.

Hossain, E., Islam, K., Yeasmin, F., Karim, M. R., Rahman, M., Agarwal, S., Hossain, S., Aziz, A., Mamun, A. A., Sheikh, A., Haque, A., Hossain, M. T., Hossain, M., Haris, P. I., Ikemura, N., Inoue, K., Miyataka, H., Himeno, S., and Hossain, K. (2012). Elevated levels of plasma Big endothelin-1 and its relation to hypertension and skin lesions in individuals exposed to arsenic. Toxicol. Appl. Pharmacol. 259, 187-194.

Hossain, M. A., Akai, J., Mihaljevič, M., Arif, M. S., Ahmed, G., Shafi, M. T., and Rahman, M. M. (2011). Arsenic contamination in groundwater of Bangladesh: perspectives on geochemical, microbial and anthropogenic issues. *Water* **3**, 1050-1076.

HSDB. (2003). Sodium Arsenate. Canadian Centre for Occupational Health and Safety. U.S. National Library of Medicine. Available at: http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB

Hu, C. W., Pan, C. H., Huang, Y. L., Wu, M. T., Chang, L. W., Wang, C. J., and Chao, M. R. (2006). Effects of arsenic exposure among semiconductor workers: a cautionary note on urinary 8-oxo-7,8-dihydro-2′-deoxyguanosine. *Free Radic. Biol. Med.* **40,** 1273-1278.

Huang, Y. K., Pu, Y. S., Chung, Y. S., Shiue, H. S., Yang, M. H., Chen, C. J., Hsueh, Y. M. (2008). Plasma folate level, urinary arsenic methylation profiles and urothelial carcinoma susceptibility. *Food Chem. Toxicol.* **46**, 929-938.

Hughes, K., Meeka, M. E., and Burnet, R. (1994). Inorganic arsenic: evaluation of risks to health from environmental exposure in Canada. *J. Environ. Sci. Health C.* **12**, 145-149.

Hughes, M. (2006). Biomarkers of exposure: a case study with inorganic arsenic. *Environ. Health Perspect.* **114**, 1790-1796.

IARC. (2004). Some drinking-water disinfectants and contaminants, including arsenic. *IARC Monographs on the evaluation of carcinogenic risks to humans*. 84: 139-141.

IARC. (1987). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Suppl. 7, Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs IARC Press, Lyon, 1 to 42:100-106.

Ishinishi, N., Kodama, Y., Nobutomo, K., and Hisanaga, A. (1997). Preliminary experimental study on carcinogenicity of arsenic trioxide in rat lung. *Environ. Health Perspect.* **19,** 191-196.

Islam, F. S., Gault, A. G., Boothman, C., Polya, D. A., Charnock, J. M., Chatterjee, D., and Lloyd, J. R. (2004). Role of metal-reducing bacteria in arsenic release from Bengal delta sediments. *Nature* **430,** 68-71.

Islam, K., Haque, A., Karim, M. R., Fajol, A., Hossain, E., Salam, K. A., Ali, N., Saud, Z. A., Rahman, M., Rahman, M., Karim, R., Sultana, P., Hossain, M., Akhand, A. A., Mandal, A., Miyataka, H., Himeno, S., and Hossain, K. (2011). Dose-response relationship between arsenic exposure and the serum enzymes for liver function tests in the individuals exposed to arsenic: a cross sectional study in Bangladesh. *Environ. Health* 10, 64.

Jha, A. N., Noditi, M., Nilsson, R., and Natarajan, A. T. (1992). Genotoxic effects of sodium arsenite on human cells. *Mutat. Res.* **284**, 215-221.

Juhasz, A. L., Smith, E., Weber, J., Rees, M., Rofe, A., Kuchel, T., Sansom, L., and Naidu, R. (2006). In vivo assessment of arsenic bioavailability in rice and its significance for human health risk assessment. *Environ. Health Perspect.* **114**, 1826-1831.

Kabata-Pendias, A., and Pendias, H. (1984). Trace Elements in Soils and Plants. CRC Press, Boca Raton, Florida. p. 315.

Karagas, M. R., Morris, J. S., Weiss, J. E., Spate, V., Baskett, C., Greenberg, E. R. (1996). Toenail samples as an indicator of drinking water arsenic exposure. *Cancer. Epidemiol. Biomarkers Prev.* **5**, 849-852.

Karagas, M. R., Tosteson, T. D., Blum, J., Klaue, B., Weiss, J. E., and Stannard, V. (2000). Measurement of low levels of arsenic exposure: a comparison of water and toenail concentrations. *Am. J. Epidemiol.***152**, 84-90.

Karim, M. R., Haque, A., Islam, K., Ali, N., Salam, K.A., Saud, Z. A., Hossain, E., Fajol, A., Akhand, A. A., Himeno, S., and Hossain, K. (2010). Interaction between chronic arsenic exposure via drinking water and plasma lactate dehydrogenase activity. *Sci. Total Environ*. **409**, 278-283.

Khan, A. W., Ahmad, S. A., Sayed, S. U., Hadi, S. A., Khan, M. H., Jalil, M. A., Ahmed, R., and Faruquee, M. H. (1997). Arsenic contamination in groundwater and its effect on human health with particular reference to Bangladesh. *J. Prevent Soc. Med.* **16,** 65-73.

Khan, M. M., Sakauchi, F., Sonoda, T., Washio, M., Mori, M. (2003). Magnitude of arsenic toxicity in tube-well drinking water in Bangladesh and its adverse effects on human health including cancer: evidence from a review of the literature. *Asian Pac. J. Cancer Prev*. **4**, 7-14.

Khan, M. M. H., Aklimunnessa, K., Kabir, M., and Mori, M. (2006). Case-control study of arsenicosis in some arsenic contaminated villages of Bangladesh. *Sapporo. Med. J.* **75,** 51-61.

Kile, M. L., Houseman, E. A., Breton, C. V., Smith, T., Quamruzzaman, Q., Rahman, M., Mahiuddin, G., and Christiani, D. C. (2007). Dietary arsenic exposure in Bangladesh. *Environ. Health Perspect.* **115**, 889-893.

Kimura, A., Ishida, Y., Hayashi, T., Wada, T., Yokoyama, H., Sugaya, T., Mukaida, N., Kondo, T. (2006). Interferon-gamma plays protective roles in sodium arsenite-induced renal injury by up-regulating intrarenal multidrug resistance-associated protein 1 expression. *Am. J. Pathol*. **169**, 1118-1128.

Kitchin, K. T. (2001). Recent advances in arsenic carcinogenesis: Modes of action, animal model systems, and methylated arsenic metabolites. *Toxicol. Appl. Pharm.***172,** 249-261.

Klassen, C. D. (2008). Casarette and Doull's Toxicology: the basic science of poisons. 7th ed. USA: Mc Graw Hill 15:936–939. Available at: http://www.ebook3000.com/Casarett-and-Doull-s-Toxicology--The-Basic-Science-of-Poisons--7th-edition--C-D-Klaassen_46972.html

Kochhar, T. S., Howard, W., Hoffman, S., Brammer-Carleton, L. (1996). Effect of trivalent and pentavalent arsenic in causing chromosome alterations in cultured Chinese hamster ovary (CHO) cells. *Toxicol. Lett*. **84**, 37-42.

Kurttio, P., Pukkala, E., Kahelin, H., Auvinen, A., and Pekkanen, J. (1999). Arsenic concentrations in well water and risk of bladder and kidney cancer in Finland. *Environ. Health Perspect.* **107**, 705-710.

Lee-Feldstein, A. (1983). Arsenic and respiratory cancer in man: Follow-up of an occupational study. *J. Natl. Cancer. Inst.* **70**, 601-610.

Lee-Feldstein, A. (1989). A comparison of several measures of exposure to arsenic. Matched case-control study of copper smelter employees. *Am. J. Epidemiol*. **129**, 112-124.

Lee, M.Y., Bae, O. N., Chung, S. M., Kang, K. T, Lee, J. Y., and Chung, J. H. (2002). Enhancement of platelet aggregation and thrombus formation by arsenic in drinking water: A contributing factor to cardiovascular disease. *Toxicol. Appl. Pharmacol.* **179**, 83-88.

Lee, T. C., Huang, R. Y., Jan, K. Y. (1985). Sodium arsenite enhances the cytotoxicity, clastogenicity and 6-thioguanine-resistant mutagenicity of ultraviolet light in Chinese hamster ovary cells. *Mutat. Res.* **148,** 83-89.

Li, D., Morimoto, K., Takeshita, T., and Lu, Y. (2001). Arsenic Induces DNA Damage via Reactive Oxygen Species in Human Cells. *Environ. Health Prev. Med.* **6,** 27-32.

Li, X., Pi, J., Li, B., Xu, Y., Jin, Y., and Sun, G. (2008). Urinary arsenic speciation and its correlation with 8-0HDG in Chinese residents exposed to arsenic through coal burning. *Bull. Environ. Contam. Toxicol.* **81**, 406-411.

Liaw, J., Marshall, G., Yuan, Y., Ferreccio, C., Steinmaus, C., and Smith, A. H. (2008). Increased childhood liver cancer mortality and arsenic in drinking water in northern Chile. *Cancer Epidemiol. Biomarkers Prev.* **17**, 1982-1987.

Libby, P. (2002). Inflammation in atherosclerosis. *Nature* **420,** 868-874.

Lindberg, A., Ekstro¨m, E. C., Nermell, B., Rahman, M., Lonnerdal, B., Persson, L. A., Vahter, M. (2007). Gender and age differences in the metabolism of inorganic arsenic in a highly exposed population in Bangladesh. *Environ. Res.* **106,** 110–120.

Lindberg, A. L., Goessler, W., Gurzau, E., Koppova, K., Rudnai, P., Kumar, R., Fletcher, T., Leonardi, G., Slotova, K., Gheorghiu, E., and Vahter, M. (2006). Arsenic exposure in Hungary, Romania and Slovakia. *J. Environ. Monit*. **8**, 203-208.

Liu, D. N., Lu, X. Z., Li, B. L., Zhou, D. X., Li, F. X., and Zheng, D. H. (1992). Clinical analysis of 535 cases of chronic arsenic poisoning from coal burning. *Chin. J. Med.* **31,** 560-562.

Liu, J., Zheng, B., Aposhian, H. V., Zhou, Y., Cheng, M. L., Zhang, A., Waalkes, M. P. (2002). Chronic arsenic poisoning from burning high-arsenic containing coal in Guizhou, China. *Environ. Health Perspect.* **110**, 119-122.

Lokuge, K. M., Smith, W., Caldwell, B., Dear, K., and Milton, A. H. (2004). The effect of arsenic mitigation interventions on disease burden in Bangladesh. *Environ. Health Perspect*. **112**, 1172-1177.

Lu, T., Liu, J., LeCluyse, E. L., Zhou, Y.-S., Cheng, M.-L., and Waalkes, M. P. (2001). Application of cDNA microarray to the study of arsenic-induced liver diseases in the population of Guizhou, China. *Toxicol. Sci.* **59**, 185-192.

Maharjan, M., Watanabe, C., Ahmad, S. A., Umezaki, M., and Ohtsuka, R. (2007). Mutual interaction between nutritional status and chronic arsenic toxicity due to groundwater contamination in an area of Terai, lowland Nepal. *J. Epidemiol. Community Health* **61**, 389-394.

Maidul, A. Z. M., Momin, A., Akramullah, S. M., Zakir, A., Afsar, S., Salahuddin, A., Tarafdar, S. A., and ALI, M. (1996). Arsenical keratoses (Chronic arsenism) - 22 cases study. *Bangladesh J. Dermatol. Venerol. Leprol.* **13**, 1- 4.

Mandal, B. K., Chowdhury, T. R., Samanta, G., Basu, G. K., Chowdhary, P. P., and Chanda, C. R. (1996). Arsenic in groundwater in seven districts of West Bengal, India–the biggest arsenic calamity in the world. *Curr. Sci.* **70**, 976-986.

Mandal, B. K., Ogra, Y., Anzai, K., and Suzuki, K. T. (2004). Speciation of arsenic in biological samples. *Toxicol. Appl. Pharmacol*. **198**, 307-318.

Mandal, B. K., Ogra, Y., and Suzuki, K. T. (2001). Identification of dimethylarsinous and monomethylarsonous acids in human urine of the arsenic-affected areas in West Bengal, India. *Chem. Res. Toxicol*. **14**, 371-378.

Mandal, B. K., and Suzuki, K. T. (2002). Arsenic round the world: a review. *Talanta* **58,** 201-235.

Marafante, E., and Vahter, M. (1987). Solubility, retention, and metabolism of intratracheally and orally administered inorganic arsenic compounds in the hamster. *Environ. Res.* **42**, 72-82.

Marchiset-Ferlay, N., Savanovitch, C., Sauvant-Rochat, M. P. (2012). What is the best biomarker to assess arsenic exposure via drinking water? *Environ. Int.* **39**, 150-171.

Mass, M. J., Tennant, A., Roop, B. C., Cullen, W. R., Styblo, M., Thomas, D. J., and Kligerman, A. D. (2001). Methylated trivalent arsenic species are genotoxic. *Chem. Res. Toxicol*. **14**, 355-361.

Mazumder, D. N. G. (2005). Effect of chronic intake of arsenic-contaminated water on liver. *Toxicol. Appl. Pharmacol.* **206,** 169-175.

Mazumder, D. N. G., Haque, R., Gosh, N., De, B. K., Santra, A., Chakraborty, D., Smith, A. H. (1998). Arsenic levels in drinking water and the prevalence of skin lesions in West Bengal, India. *Int. J. Epidemio.* **27,** 871–877.

Mazumder, D. N. G., Haque, R., Ghosh, N., De, B. K., Santra, A., Chakraborti, D., and Smith, A. H. (2000). Arsenic in drinking water and the prevalence of respiratory effects in West Bengal, India. *Int. J. Epidemiol.* **29,** 1047-1052.

Meharg, A. A. (2004). Arsenic in rice – understanding a new disaster for South-East Asia. *Trends. Plant Sci.* **9,** 415-417.

Meharg, A. A., Deacon, C., Campbell, R. C., Carey, A. M., Williams, P. N., Feldmann, J., and Raab, A. (2008). Inorganic arsenic levels in rice milk exceed EU and US drinking water standards. *J. Environ. Monit.* **10,** 428-431.

Meharg, A. A., and Raab, A. (2010). Getting to the bottom of arsenic standards and guidelines. *Environ. Sci. Technol*. **44**, 4395-4399.

Meharg, A. A., and Rahman, M. M. (2003). Arsenic contamination of Bangladesh paddy field soils: implications for rice contribution to arsenic consumption. *Environ. Sci. Technol.* **37**, 229–334.

Meharg, A. A., and Zhao, F. J. (2012). Arsenic & Rice, London: Springer.

Meliker, J. R., Wahl, R. L., Cameron, L. L., Nriagu, J. O. (2007). Arsenic in drinking water and cerebrovascular disease, diabetes mellitus, and kidney disease in Michigan: a standardized mortality ratio analysis. *Environ. Health* **2,** 4-6.

Meza, M. M., Kopplin, M. J., Burgess, J. L, Gandolfi, A. J. (2004). Arsenic drinking water exposure and urinary excretion among adults in the Yaqui Valley, Sonora, Mexico. *Environ. Res*. **96**, 119-126.

Michaud, D. S., Wright, M. E., Cantor, K. P., Taylor, P. R., Virtamo, J., and Albanes, D. (2004). Arsenic concentrations in prediagnostic toenails and the risk of bladder cancer in a cohort study of male smokers. *Am. J. Epidemiol*. **160**, 853-859.

Miller, W. H. Jr., Schipper, H. M., Lee, J. S., Singer, J., Waxman, S. (2002). Mechanisms of action of arsenic trioxide. *Cancer Res*. **62**, 3893-903.

Milton, A. H., Hasan, Z., Rahman, A., and Rahman, M. (2003). Non-cancer effects of chronic arsenicosis in Bangladesh: preliminary results. *J. Environ. Sci. Health A. Tox. Hazard Subst. Environ. Eng.* **38**, 301-305.

Milton, A. H., Hasan, Z., Shahidullah, S. M., Sharmin, S., Jakariya, M. D., Rahman, M., Dear, K., and Smith, W. (2004). Association between nutritional status and arsenicosis due to chronic arsenic exposure in Bangladesh. *Int. J. Environ. Health Res.* **14**, 99-108.

Milton, A. H., and Rahman, M. (2002). Respiratory effects and arsenic contaminated well water in Bangladesh. *Int. J. Environ. Health Res*. **12,** 175-179.

Milton, A.H., Smith, W., Rahman, B., Hasan, Z., Kulsum, U., Dear, K., Rakibuddin, M., Ali, A. (2005). Chronic arsenic exposure and adverse pregnancy outcomes in Bangladesh. *Epidemiology* 16, 82-86.

Mondal, D., and Polya, D. A. (2008). Rice is a major exposure route for arsenic in Chakdaha block, Nadia district, West Bengal, India: A probabilistic risk assessment. *Appl. Geochem.* **23**, 2987-2998.

Moon, C., Marlowe, M., Stellern, J., 2007, Errera, J. (1985). Main and interaction effects of metallic pollutants on cognitive functioning. *J. Learn. Disabil.* **18**, 217–221.

Morton, W, E., and Caron, G. A. (1989). Encephalopathy: An uncommon manifestation of workplace arsenic poisoning. *Am. J. Ind. Med.* **15**, 1-5.

Mosaferi, M., Yunesian, M., Dastgiri, S. Mesdaghinia, A., and Esmailnasab, N. (2008). Prevalence of skin lesions and exposure to arsenic in drinking water in Iran. *Sci. Total Environ.* **390**, 69-76.

Mukherjee, A., Sengupta, M. K., Hossain, M. A., Ahamed, S., Das, B., Nayak, B., Lodh, D., Rahman, M. M., and Chakraborti, D. (2006). Arsenic contamination in groundwater: a global perspective with emphasison the Asian scenario. *J. Health Popul. Nutr.* **24**, 142-163.

Mumford, J. L., Wu, K., Xia, Y., Kwok, R., Yang, Z., Foster, J., and Sanders, W. E. (2007). Chronic Arsenic Exposure and Cardiac Repolarization Abnormalities with

QT Interval Prolongation in a Population-based Study. *Environ. Health Perspect.* **115**, 690-694.

Murukesh, N., Dive, C., and Jayson, G. C. (2010). Biomarkers of angiogenesis and their role in the development of VEGF inhibitors. *Br. J. Cancer* **102**, 8-18.

Ng, J. C., Wang, J., and Shraim, A. (2003). A global health problem caused by arsenic from natural sources. *Chemosphere* **52,** 1353-1359.

Nickson, R. T., McArthur, J. M., Burgess, W. G., Ahmed, K. M., Ravenscroft, P., and Rahman, M. (1998). Arsenic poisoning of Bangladesh groundwater. *Nature* **395**, 338.

Nicolli, H. B., Suriano, J. M., and Gomez, P. (1989). Groundwater contamination with arsenic and other trace elements in an area of the Pampa, Province of Cordoba, Argentina. *Environ. Geol. Water. Sci.* 14, 3-16.

Nordstrom, D. K. (2002). Worldwide occurences of arsenic in ground water. *Science* **296**, 2143-2145.

NRC. (1999). Subcommittee on Arsenic in Drinking Water. Arsenic in Drinking Water. National Academy press; Washington, DC. Available at: http://www.nap.edu/openbook.php?isbn=0309063337

NRC (2001). Arsenic in Drinking Water: 2001 Update. National Academy press, Washington DC.
Available at: http://www.nap.edu/catalog.php?record_id=10194

Nriagu, J. O., and Azcue, J. M. (1990). In: Nriagu JO (Ed.), Arsenic in the environment. Part 1: Cycling and characterization, John Wiley and Sons, Inc, New York, pp.1-15.

Okui, T., and Fujiwara, Y. (1986). Inhibition of human excision DNA repair by inorganic arsenic and the co-mutagenic effect in V79 Chinese hamster cells. *Mutat. Res.* **172**, 69-76.

Onishi, H. Arsenic, in: K.H. Wedepohl (Ed.), Handbook of Geochemistry, Springer-Verlag, New York, 1969, Vol. II-2, Chapter 33.

Paffen, E., and deMaat, M. P. M. (2006). C-reactive protein in atherosclerosis: A causal factor? *Cardiovasc. Res*. **71**, 30-39.

Pant, N., Kumar, R., Murthy, R. C., and Srivastava, S. P. (2001). Male reproductive effect of arsenic in mice. *Biometals*. 14, 113-117.

Parrish, A. R., Zheng, X. H., Turney, K. D., Younis, H. S., and Gandolfi, A. J. (1999). Enhanced transcription factor DNA binding and gene expression induced by arsenite or arsenate in renal slices. *Toxicol. Sci.* **50,** 98-105.

Peterson, P. J., Benson, L. M., Zeive, R. (1981). Metalloids, in: Lepp MW (Ed.), Arsenic and effect of heavy metal pollution on plants, vol. 1, Appl. Sci. Publ, London, 1981, p. 299.

Piao, F., Ma, N., Hiraku, Y., Murata, M., Oikawa, S., Cheng, F., Zhong, L., Yamauchi, T., Kawanishi, S., and Yokoyama, K. (2005). Oxidative DNA damage in relation to neurotoxicity in the brain of mice exposed to arsenic at environmentally relevant levels. *J. Occup. Health* **47,** 445-449.

Polissar, L., Lowry-Coble, K., Kalman, D. A., Hughes, J. P., van Belle, G., Covert, D. S., Burbacher, T. M., Bolgiano, D., and Mottet, N. K. (1990). Pathways of human exposure to arsenic in a community surrounding a copper smelter. *Environ. Res.* **53,** 29-47.

Polizzotto, M. L., Harvey, C. F., Li, G., Badruzzman, B., Ali, A., Newville, M., Sutton, S., and Fendorf, S. (2006). Solid-phases and desorption processes of arsenic within Bangladesh sediments. *Chem. Geol.* **228,** 97-111.

Pomroy, C., Charbonneau, S. M., Mccullough, R. S., and Tam, G. K. (1980). Human retention studies with 74As. *Toxicol. Appl. Pharmacol.*, **53**, 550-556.

Rahman, A., Vahter, M., Ekstrom, E. C., Rahmanm, H., Golam Mustafa, A. H., Wahed, M. A., Yunus, M., and Persson, L. A. (2007). Association of arsenic exposure during pregnancy with fetal loss and infant death: a cohort study in Bangladesh. *Am. J. Epidemiol.* **165**, 1389-1396.

Rahman, M., Saud, Z. A., Hossain, E., Islam, K., Karim, M. R., Hoque, M. M., Yeasmin, T., Nikkon, F., Mandal, A., and Hossain, K. (2012). The ameliorating effects of *Zingiber zerumbet* Linn. on sodium arsenite-induced changes of blood indices in experimental mice. *Life Sci. Med. Res.* LSMR-41.

Rahman, M., Tondel, M., Ahmad, S. A., Chowdhury, I. A., Faruquee, M. H., and Axelson, O. (1999). Hypertension and arsenic exposure in Bangladesh. *Hypertension* **33**, 74-78

Rahman, M. A., Hasegawa, H., Rahman, M. M., Miah, M. A. M., and Tasmin, A. (2008). Arsenic accumulation in rice (*Oryza sativa* L.): Human exposure through food chain. *Ecotoxicol. Environ. Saf.* **69,** 317-324.

Rahman, M. M., Chowdhury, U. K., Mukherjee, S. C., Mondal, B. K., Paul, K., Lodh, D., Biswas, B. K., Chanda, C. R., Basu, G. K., Saha, K. C., Roy, S., Das, R., Palit, S. K., Quamruzzaman, Q., and Chakraborti, D. (2001). Chronic arsenic toxicity in Bangladesh and West Bengal, India, a review and commentary. *J. Toxicol. Clin. Toxicol.***39,** 683-700.

Rahman, M. M., Sengupta, M. K., Ahamed, S., Chowdhury, U. K., Lodh, D., Hossain, A., Das, B., Roy, N., Saha, K. C., Palit, S. K., and Chakraborti, D. (2005). Arsenic contamination of groundwater and its health impact on residents in a village in West Bengal, India. *Bull. World Health Organ.* **83,** 49-57.

Rasmussen, R. E., and Menzel, D. B. (1997). Variation in arsenic-induced sister chromatid exchange in human lymphocytes and lymphoblastoid cell lines. *Mutat. Res*. **386,** 299-306.

Razo, L. M. D., Arellano, M. A., and Cebrian, M. E. (1990). The oxidation states of arsenic in well-water from a chronic arsenicism area of Northern Mexico. *Environ. Pollut.***64,**143-153.

Rodríguez, V. M., Carrizales, L., Mendoza, M. S., Fajardo, O. R., and Giordano, M. 2002. Effects of sodium arsenite exposure on development and behavior in the rat. *Neurotoxicol. Teratol*. **24**, 743-750.

Ross, R. (1999). Atherosclerosis is an inflammatory disease. *Am. Heart. J.* **138**, 419-420.

Rossman, T. G., Uddin, A. N., and Burns, F. J. (2004). Evidence that arsenite acts as a cocarcinogen in skin cancer. *Toxicol. Appl. Pharmacol*. **198,** 394-404.

Roychowdhury, T., Uchino, T., and Tokunaga, H. (2008). Effect of arsenic on soil, plant and foodstuffs by using irrigated groundwater and pond water from Nadia district, West Bengal. *Int. J. Environ. Pollut.* **33,** 218-234.

Safiullah, S. (2006). Arsenic pollution in the groundwater in Bangladesh; an overview. *Asian. J. Water Environ. Pollut.* **4,** 47-59.

Saha, K. C. (1995). Chronic arsenical dermatoses from tube-well water in West Bengal during 1983-87. *Indian J. Dermatol.* **40**, 1-12.

Saha, K. K., Engström, A., Hamadani, J. D., Tofail, F., Rasmussen, K. M., and Vahter, M. (2012). *Environ. Health Perspect*. **120**, 1208-1214.

Sancha, A. M., and Castro, M. L. (2001). In: Cambell WR, Abernathy CO, Calderon RL, editors. Arsenic: exposure and health effects IV. Amsterdam: Elsevier, 87-96.

Santra, A., Das Gupta, J., De, B. K., Roy, B., and Mazumder, DNG. (1999). Hepatic manifestations in chronic arsenic toxicity. *Indian J. Gastroenterol.* **18**, 152-155.

Scott, M. G., Ivor, J. B., Gregory, L. B., Alan, C., Robert, H. E., Barbara, V. H., Mitch,W., Sidney C. S., and James, R. S. (1999. Diabetes and cardiovascular disease: a statement for healthcare professionals from the American Heart Association. *Circulation* **100**, 1134-1146.

Shalat, S. L., Walker, D. B., and Finnell, R. H. (1996). Role of arsenic as a reproductive toxin with particular attention to neural tube defects. *J. Toxicol. Environ. Health* **48,** 253-272.

Shannon, R. L., and Strayer, D. S. (1989). Arsenic-induced skin toxicity. *Hum. Toxicol.* **8**, 99-104.

Shibata, Y., Yoshinage, J., and Morita, M. (1994). Detection of arsenobetaine in human blood. *Appl. Organometal. Chem.* **8**, 249-251.

Slejkovec, Z., Falnoga, I., Goessler, W., van Elteren, J., Raml, R., Podgornik, H., and Cernelc, P. (2008). Analytical artefacts in the speciation of arsenic in clinical samples. *Anal. Chim. Acta.* **607**, 83-91.

Slotnick, M. J., and Nriagu, J. O. (2006). Validity of human nails as a biomarker of arsenic and selenium exposure: a review. *Environ. Res.***102**, 125-139.

Smith, A. H. (1998). Technical report and review of action plan for arsenic in drinking water in Bangladesh focusing on health. Assignment Report, WHO Project BAN CWS 001/D, February 1998. Available at http://socrates. berkeley.edu/~asrg/).

Smith, A. H., Lingas, E. O., and Rahman, M. (2000). Contamination of drinking-water by arsenic in Bangladesh: a public health emergency. *Bull. World Health Organ.* **78**, 1093-1103.

Sohel, N., Persson, L. A., Rahman, M., Streatfield, P. K., Yunus, M., Ekstrom, E. C., and Vahter, M. (2009). Arsenic in drinking water and adult mortality: a population-based cohort study in rural Bangladesh. *Epidemiology* **20**, 824-830.

Steinberg, D., and Witztum, J. L. (2002). Is the oxidative modification hypothesis relevant to human atherosclerosis? Do the antioxidant trials conducted to date refute the hypothesis? *Circulation* **105**, 2107-2111.

Straub, A. C., Stolz, D. B., Ross, M. A., Hernandez-Zavala, A., Soucy, N. V., Klei, L. R., and Barchowsky, A. (2007). Arsenic stimulates sinusoidal endothelial cell capillarization and vessel remodeling in mouse liver. *Hepatology* **45**, 205-212.

Sun, G. (2004). Arsenic contamination and arsenicosis in China. *Toxicol. Applied Pharmacol.* **198,** 268-271.

Sun, G., Xu, Y., Li, X., Jin, Y., Li, B., and Sun, X. (2007). Urinary arsenic metabolites in children and adults exposed to arsenic in drinking water in Inner Mongolia, China. *Environ. Health Perspect*. **115**, 648-652.

Sun, G. X., Williams, P. N., Carey, A. M., Zhu, Y. G., Deacon, C., Raab, A., Feldmann, J., Islam, R. M., and Meharg, A. A. (2008). Inorganic arsenic in rice bran and its products are an order of magnitude higher than in bulk grain. *Environ. Sci. Technol.* **42**, 7542-7546.

Sutton, N. B., van der Kraan, G. M., van Loosdrecht, M. C. M., Bruining, G. M. J., and Schotting, R. J. (2009). Characterization of geochemical constituents and bacterial populations associated with As mobilization in deep and shallow tube wells in Bangladesh. *Water Res.***43**, 1720-1730.

Tamaki, S., and Frankenberger, W. T. Jr. (1992). Environmental biochemistry of arsenic. *Rev. Environ. Contam. Toxicol.* **124**, 79-110.

Tapio, S. and Grosche, B. (2006). Arsenic in the aetiology of cancer. *Mutat. Res.* **612**, 215-246.

Tchounwou, P. B., Patlolla, A. K., and Centeno, J. A. (2003). Carcinogenic and systemic health effects associated with arsenic exposure-a critical review. *Toxicol. Pathol*. **31**,575-588.

Trenary, H. R., Creed, P. A., Young, A. R., Mantha, M., Schwegel, C. A., Xue, J., Kohan, M. J., Herbin-Davis, K., Thomas, D. J., Caruso, J. A., and Creed, J. T. (2012). An in vitro assessment of bioaccessibility of arsenicals in rice and the use of this estimate within a probabilistic exposure model. *J. Expo. Sci. Environ. Epidemiol.* **22**, 369-375.

Tsai, S. M., Wang, T. N., and Ko, Y. C. (1999). Mortality for certain diseases in areas with high level of arsenic in drinking water. *Arch. Environ. Health.* **54**, 186-193.

Tseng, C. H. (1999). Chronic arsenic intoxication in Asia: Current perspectives. *J Intern. Med. Taiwan* **10**, 224-229.

Tseng, C. H. (2007). Arsenicmethylation, urinary arsenic metabolites and human diseases: current perspective. *J. Environ. Sci. Health C. Environ. Carcinog. Ecotoxicol. Rev.* **25**, 1-22.

Tseng, C. H., Chong, C. K., Chen, C. J., and Tai, T. Y. (1996). Dose–response relationship between peripheral vascular disease and ingested arsenic among residents in blackfoot disease endemic villages in Taiwan. *Atherosclerosis* **120,** 125-133.

Tseng, C. H., Chong, C. K., Tseng, C. P., Hsueh, Y. M., Chiou, H. Y., Tseng, C. C., and Chen, C. J. (2003). Long-term arsenic exposure and ischemic heart disease in arseniasis-hyperendemic villages in Taiwan. *Toxicol. Lett.* **127,** 15-21.

Tseng, W. P. (1977). Effect of dose-response relationships of skin cancer and Blackfoot disease with arsenic. *Environ. Health Perspect.* **19,** 109-119.

Tseng, W. P., Chu, H. M., How, S. W., Fong, J. M., Lin, C. S., and Yeh, S. (1968). Prevalence of skin cancer in an endemic area of chronic arsenism in Taiwan. *J. Natl. Cancer Inst.* **40,** 453-463.

Ullah, S. M. (1998). Arsenic contamination of groundwater and irrigated soils of Bangladesh. In Abstracts: Int. Conf. Arsenic Pollution of Groundwater in Bangladesh: Causes, Effects and Remedies, 8-12 February 1998, DCH, Dhaka, P.133.

Vahidnia, A., Romijn, F., Tiller, M., van der Voet, G. B., and de Wolff, F. A. (2006). Arsenic-induced toxicity: effect on protein composition in sciatic nerve. *Hum. Exp. Toxicol*. **25**, 667-674.

Vahidnia, A., Romijn, F., van der Voet, G. B., and de Wolff, F. A. (2008). Arsenic-induced neurotoxicity in relation to toxicokinetics: Effects on sciatic nerve proteins. *Chem. Biol. Interact.* **176**, 188-195.

Vahter, M. (2002). Mechanisms of arsenic biotransformation. *Toxicology* **181-182,** 211-217.

Vahter, M., Chappell, W. R., Abernathy, C. O., and Calderon, R. L. (1999). Variation in human metabolism of arsenic. Arsenic exposure and health effects. Oxford: Elsevier Science Ltd. p. 267-279.

Vahter, M., and Marafante, E. (1987). Effects of low dietary intake of methionine, choline or proteins on the biotransformation of arsenite in the rabbit. Toxicol. Lett. 37, 41-46.

Valko, M., Morris, H., and Cronin, M. T. (2005). Metals, toxicity and oxidative stress. *Curr. Med. Chem*. **12**, 1161-1208.

Vinogradov, A. P. (1959). The Geochemistry of Rare and Dispersed Chemical Elements in Soils, 2nd ed., New York, p. 65.

von Ehrenstein, O. S., Mazumder, D. N. G., Hira-Smith, M., Ghosh, N., Yuan, Y., Windham, G., Ghosh, A., Haque, R., Lahiri, S., Kalman, D., Das, S., and Smith, A. H. (2006). Pregnancy outcomes, infant mortality, and arsenic in drinking water in West Bengal, India. *Am. J. Epidemiol*. **163**, 662-669.

Vuyyuri, S. B., Ishaq, M., Kuppala, D., Grover, P., and Ahuja, Y. R. (2006). Evaluation of micronucleus frequencies and DNA damage in glass workers exposed to arsenic. *Environ. Mol. Mutagen* **47**, 562-570.

Wang, C. H., Hsiao, C. K., Chen, C. L., Hsu, L. I., Chiou, H. Y., Chen, S. Y., Hsueh, Y. M., Wu, M. M., and Chen, C. J. (2007). A review of the epidemiologic literature on the role of environmental arsenic exposure and cardiovascular diseases. *Toxicol. Appl. Pharmacol*. **222**, 315-326.

Wang, C. H., Jeng, J. S., Yip, P. K., Chen, C. L., Hsu, L. I., Hsueh, Y. M., Chiou, H. Y., Wu, M. M., and Chen, C. J. (2002). Biological gradient between long-term arsenic exposure and carotid atherosclerosis. *Circulation* **105**, 1804-1809.

Watanabe, C., Inaoka, T., Kadono, T., Nagano, M., Nakamura, S., Ushijima, K., Murayama, N., Miyazaki, K., and Ohtsuka, R. (2001). Males in Rural Bangladeshi Communities Are More Susceptible to Chronic Arsenic Poisoning than Females: Analyses Based on Urinary Arsenic. *Environ. Health Perspect.* **109**, 1265-1270.

Welch, A. H., Lico, M. S., and Hughes, J. L. (1988). Arsenic in ground water of the western United States. *Groundwater* **26**, 333-347.

WHO. (1981). Environmental Health Criteria 18, Arsenic: World Health Organisation, Geneva.

WHO. (1996). Geneva. Guidelines for Drinking Water Quality, Recommendations. 2nd ed., vol. 2.

WHO. (2000). Towards an Assessment of the Socioeconomic Impact of Arsenic Poisoning in Bangladesh. WHO/SDE/WSH/00.4, WHO, Geneva. Available at: http://www.bvsde.ops-oms.org/bvsaca/i/fulltext/impact/impact.pdf

WHO. (2001). Environmental Health Criteria 224, 2nd ed., Arsenic Compounds: World Health Organisation, Geneva.

Wiencke, J. K., and Yager, J. W. (1991). Specificity of arsenite in potentiating cytogenetic damage induced by the DNA crosslinking agent diepoxybutane. *Environ. Mol. Mutagen* **19**, 195-200.

Winski, S. L., and Carter, D. E. (1995). Interactions of rat red blood cell sulfhydryls with arsenate and arsenite. *J. Toxicol. Environ. Health* **46**, 379-397.

Wu, M. M., Kuo, T. L., Hwang, Y. H., and Chen, C. J. (1989). Dose–response relation between arsenic concentration in well water and mortality from cancers and vascular diseases. *Am. J. Epidemiol*. **130**, 1123-1132.

Yamanaka, K., Kato, K., Mizoi, M., An, Y., Takabayashi, F., Nakano, M., Hoshino, M., and Okada, S. (2004). The role of active arsenic species produced by metabolic reduction of dimethylarsinic acid in genotoxicity and tumorigenesis. *Toxicol. Appl. Pharmacol*. **198**, 385-393.

Yamauchi, H., and Fowler, B. A. (1994). Toxicity and metabolism of inorganic and methylated arsenicals. In: Nriagu J.O., ed. Arsenic in the environment, Part II: Human health and ecosystem effects. New York, John Wiley & Sons., 35-53.

Yip, S. F., Yeung, Y. M., and Tsui, E. Y. (2002). Severe neurotoxicity following arsenic therapy for acute promyelocytic leukemia: potentiation by thiamine deficiency. *Blood* **99**, 3481-3482.

Yoshida, T., Yamauchi, H., and Fan, S. G. (2004). Chronic health effects in people exposed to arsenic via the drinking water: dose-response relationships in review. *Toxicol. Appl. Pharmacol.* **198**, 243-252.

Yuan, Y., Marshall, G., Ferreccio, C., Steinmaus, C., Selvin, S., Liaw, J., Bates, M. N., and Smith, A. H. (2007). Acute myocardial infarction mortality in comparison with lung and bladder cancer mortality in arsenic-exposed region II of Chile from 1950 to 2000. *Am. J. Epidemiol*. **166**, 1381-1391.

Yu, R. C., Hsu, K. H., Chen, C. J., and Froines, J. R. (2000). Arsenic methylation capacity and skin cancer. *Cancer Epidemiol. Biomarkers Prev*. **9**, 1259-1262.

Zhao, C. Q., Young, M. R., Diwan, B. A., Coogan, T. P., and Waalkes, M. P. (1997). Association of arsenic-induced malignant transformation with DNA hypomethylation and aberrant gene expression. *Proc. Natl. Acad. Sci. USA.* **94**, 10907-10912.

Zheng, Y., Stute, M., van Geenb, A., Gavrieli, I., and Dhara, R. (2004). Redox control of arsenic mobilization in Bangladesh groundwater. *Appl. Geochem.* **19**, 201–214.

Zuane, J. D. (1990). Handbook of Drinking Water Quality, Standards and Controls, Van Nostrand Reinhold.

Chapter 2: Exploring the association of arsenic exposure with inflammatory and adhesion molecules related to atherosclerosis in the individuals exposed to arsenic in Bangladesh

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2. Abstract

Exposure to elevated levels of arsenic through contaminated drinking water is a global health problem. Arsenic exposure increases mortality caused by cardiovascular diseases (CVDs). Atherosclerosis is the most common pathologic process underlying CVDs. Several inflammatory and adhesion molecules are closely related with the biochemical event of atherosclerosis. However, association of arsenic exposure with inflammatory and adhesion molecules have not yet been fully documented. This study has been designed to explore the association of arsenic exposure with plasma levels of inflammatory molecule C-reactive protein (CRP) and adhesion molecules including intercellular adhesion molecule-1(ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) in the individual who exposed to arsenic chronically in Bangladesh. A total of 324 study subjects, 218 from arsenic-endemic areas and 106 from non-endemic area, were recruited. Arsenic concentrations in water, hair and nails were measured by Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) and plasma levels of CRP, ICAM-1 and VCAM-1 were quantified using respective immunoassay kits through microplate reader. Plasma levels of CRP, ICAM-1 and VCAM-1 in the arsenic-endemic areas were higher than those of non-endemic area. We found that plasma CRP, ICAM-1 and VCAM-1 levels were increased with the increasing concentrations of arsenic in water, hair and nails. Further, to evaluate the dose-response relationship between arsenic exposure metrics and plasma levels of these molecules; the study subjects were split into quartile groups based on four different concentrations of arsenic in water, as well as in hair and nails where study subjects in the non-endemic area were used as a lowest (reference) exposure group. Plasma CRP, ICAM-1 and VCAM-1 levels were found to be higher in the higher exposure groups as compared to the lowest exposure group. Associations of plasma CRP, ICAM-1 and VCAM-1 levels with arsenic exposure metrics remain significant even after adjustment with relevant covariate such as age, sex, BMI, smoking, hypertension, occupation, education and monthly income. Thus, all these results explicitly suggest that arsenic exposure increase the circulating inflammatory and adhesion molecules that are implicated in atherosclerosis.

2.1 Introduction

Chronic exposure to arsenic through drinking water is a major public health hazards in certain parts of the world including Bangladesh. Arsenic toxicity through drinking water represents one of the biggest catastrophes in history, affecting millions of people in the world. Bangladesh is one of the most severely affected regions in that approximately 80 million people consume water containing arsenic levels greater than the 10 μg/L standard set by World Health Organization (WHO) (Caldwell et al., 2003; Chowdhury A, 2004; Chowdhury U et al., 2000). There is great temporal and spatial variation in groundwater arsenic levels in different regions of Bangladesh. Arsenic is widely present in natural waters, in the form of inorganic arsenite (As^{III}) and arsenate (As^{V}). After consumption, inorganic arsenic is converted to methylated derivatives. Although methylation of arsenic has been commonly considered a mechanism for detoxification, recent studies have shown that methylated trivalent arsenicals are more toxic than inorganic arsenic (Kligerman et al., 2003). Still there are no appropriate animal models available for investigating health effects of arsenic. Therefore, significant uncertainties remain in the mechanisms of the health effects of human exposure to arsenic.

Cardiovascular diseases (CVDs) are the major causes of arsenic-related morbidity and mortality (Chen et al., 2011; Navas-Acien et al., 2005; Wang et al., 2007). Clinical manifestations of arsenic-induced CVDs include hypertension, coronary heart disease, stroke, and peripheral arterial diseases (Rahman et al., 1999; Tseng et al., 2003; Wang et al., 2002, 2009). Atherosclerosis is the central event of CVDs. Atherosclerosis is mainly caused by hardening of the arteries due to a thickening of the artery lining from fatty deposits or plaques (atheroma). It is now well understood that atherosclerosis is a multifactorial pathophysiological process. The blood-circulating molecules including lipoproteins, inflammatory and adhesion molecules have been reported to be involved in the formation of atherosclerotic lesions and many of them are predictive for atherosclerosis and CVDs (Blankenberget al., 2001; Hwang et al., 1997).

Recent reports suggest that inflammation has a key role in the pathogenesis of atherosclerosis as well as CVDs (Libby 2002; Ross 1999; Steinberg 2002). C-reactive protein (CRP) is an acute-phase protein produced mainly by the liver and also by adipocytes and vascular smooth muscle cells in response to interleukin-6 (IL-6), IL-1,

and tumor necrosis factor-alpha (TNF-α) (Calabro et al., 2003; Castell et al., 1990; Madjid et al., 2007, Tilg et al., 1992). Among other inflammatory markers CRP has emerged as the most powerful inflammatory predictor of future cardiovascular risk (Haverkate, 1997; Hirschfield and Pepys, 2003; Paffen et al., 2006; Ridker, 2000). Many clinical studies have demonstrated CRP as an independent predictor of atherosclerosis, cardiovascular events, atherothrombosis, hypertension and myocardial infarction, even after considering other cardiovascular risk factors such as age, smoking, obesity, diabetes, hypercholesterolemia and hypertension (Black et al., 2004; Libby and Ridker, 2004; Pepys and Hirschfield, 2001; Sesso et al., 2003). CRP coupled with lipoproteins is found in atherosclerotic plaques and all acute myocardial infarction lesions. Further, CRP can directly interact with atherosclerotic vessels by activating complement system that can ultimately promote inflammation and thrombosis (Lagrand et al., 2002). Recently, animal model studies have suggested that arsenic treatment causes the elevation of CRP (Cheng et al., 2011; Druwe et al., 2012). However, no human data are available in this regards.

Circulating adhesion molecules have been shown to be associated with increased risk of cardiovascular events (Mulvihill et al., 2002; Ridker et al., 1998). Intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), are endothelial adhesion molecules of the immunoglobulin gene superfamily that play important role in inflammation, immune responses and in intracellular signalling events (Gahmberg et al., 1997). ICAM-1 and VCAM-1 have been recognized as markers of endothelial dysfunction. They are mainly expressed and secreted by the vascular endothelial cells and circulating leukocytes in response to inflammatory stimulants. ICAM-1 and VCAM-1 participate in atherogenesis by mediating the inflammatory cells recruitment from the circulation and their transendothelial migration to the sites of inflammation, a crucial step for initiation and progression of atherosclerosis (Libby, 2002; Ross, 1999; Springer, 1994). Elevated levels of circulating ICAM-1 and VCAM-1 have been reported to be associated with cardiovascular risk (Blankenberg et al., 2001; Hope and Meredith, 2003; Hwang et al., 1997; Ridker et al., 1998, 2000; Rohde et al., 1998; Schmidt et al., 2009; Simundic et al., 2004).

Although many epidemiological studies have shown that arsenic exposure increases the risk of atherosclerosis, but limited information is available on the changes in biochemical markers for atherosclerosis in individuals exposed to arsenic. Therefore, the aim of this study was to investigate the effects of arsenic exposure on the circulating inflammatory and adhesion molecules related to atherosclerosis in the individuals exposed to arsenic in Bangladesh.

2.2 Materials and Methods

2.2.1 Ethical permission

Ethical permission was obtained from the Institute of Biological Sciences, University of Rajshahi, Bangladesh (21/320-IAMEBBC/IBSc). The subjects who participated in this study gave their written consent. All sorts of confidentialities and rights of the study subjects were strictly maintained.

2.2.2 Selection of study areas and subjects

The arsenic-endemic and non-endemic areas and study subjects were selected as described previously (Ali et al., 2010; Hossain et al., 2012; Karim et al., 2010). Arsenic-endemic study areas were selected from the North-West region of Bangladesh that included Marua in Jessore, Dutpatila, Jajri, Vultie and Kestopur in Chuadanga and Bheramara in Kushtia districts. Chowkoli, a village in Naogaon district with no history of arsenic contamination was selected as non-endemic area. Arsenic-endemic study areas were chosen from the north-west region of Bangladesh according to the British Geological Survey report (2001). However, more detailed information on the location of the endemic-villages was taken from the local health offices. We found that all families did not have their own drinking water (tube well water) sources. In many cases, a group of families used drinking water from one tube well. We recruited our study subjects based on the largest possible number of family members or families who used water from the selected tube wells. Many local residents had typical skin symptoms of arsenicosis (melanosis, hyperkeratosis, and hard patches on the palms of the hands and soles of the feet). Study subjects were selected irrespective of their skin symptoms. Those who responded spontaneously were asked to convene at a specific location in their village for initial screening purpose in light of exclusion criteria. The adults (15 to 60 years of ages) who had lived for at least last five years in the arsenic-endemic and non-endemic areas were

recruited for this study. During the sample collection process, we were blinded to arsenic levels in drinking water, and to those in the hair and nails of the study participants. Attempt was made to match, as much as possible the following: age, sex and socioeconomic parameters (occupation, monthly income and education) of arsenic-endemic and non-endemic study subjects. The ratios of endemic and non-endemic subjects were approximately 2:1, and male and female ratios in both endemic and non-endemic areas were also approximately 1:1. Endemic and non-endemic study subjects were individually matched on age (± 5 years). Further, both endemic and non-endemic study subjects were villagers and other socioeconomic parameters such as occupation, monthly income and education levels were almost closely matched.

Pregnant and lactating women, Hepatitis B positive, and the individuals who had a history of drug addiction, chronic alcoholism, prescription of hepatotoxic and anti-hypertensive drugs, malaria, kalazar, and hepatic, renal or cardiac diseases were excluded from this study. Of the 225 individuals recruited in arsenic-endemic areas, seven individuals were excluded according to the above-mentioned criteria. In non-endemic area, four individuals were excluded from the 110 individuals recruited. Thus, the numbers of participants from arsenic-endemic and non-endemic areas were 218 and 106, respectively.

The interview of the study subjects was carried out by the trained members of the research team by visiting each household and using a standard questionnaire. Information obtained from the interview included the sources of water for drinking and daily house hold uses, water consumption history, socioeconomic status, occupation, food habit, cigarette smoking, alcohol intake, personal and family medical history, history of diseases, physiological complications, major diseases, previous physician's reports, and body mass index (BMI).

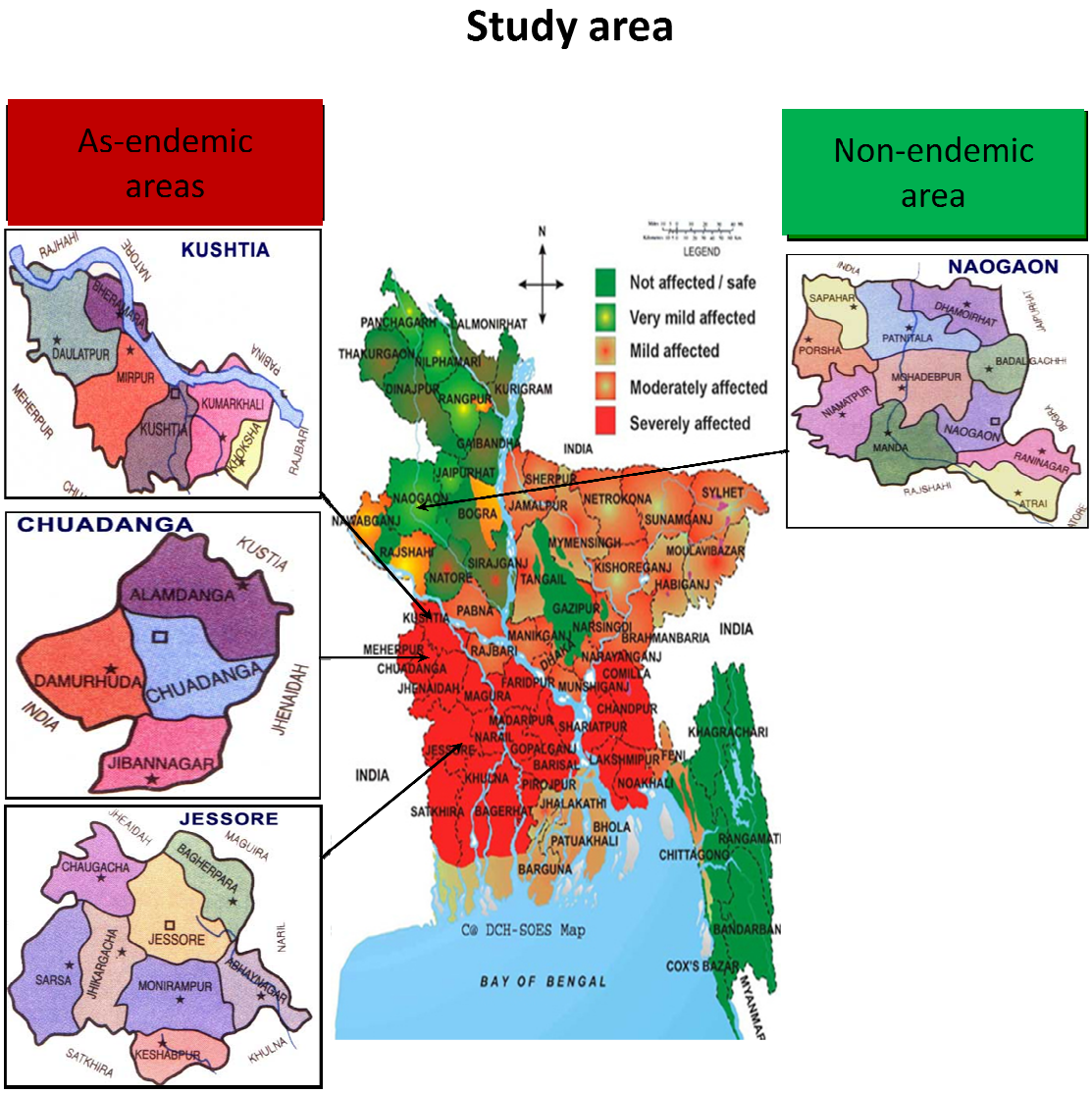

Figure 2.1 Arsenic-endemic (left panel) and non-endemic (right panel) areas in Bangladesh selected for this study.

Source: Groundwater Arsenic condition in GMB Plain at International Arsenic Conference (1998) by SOES-DCH at Dhaka, Bangladesh.
Available at: http://www.soesju.org/arsenic/alb_37.html

2.2.3 Water collection and arsenic analysis

Water samples were collected from the tube wells which the study subjects used as a primary source of drinking water as described previously (Ali et al., 2010). Water samples were collected on the same day when hair, nail, and blood samples of the study subjects were collected. After the tube well was pumped for five minutes, the water samples from these tube wells were collected in acid-washed containers (van

Geen et al., 2008). Total arsenic concentration in water samples was determined by Inductively Coupled Plasma Mass Spectroscopy (ICP-MS, HP-4500, Agilent Technologies, Kanagawa, Japan) after the addition of a solution of yttrium (10 µg/L in 1.0% nitric acid) as an internal standard for ICP-MS analysis. All samples were determined in triplicate and the average values were used. Accuracy of ICP-MS determination of water arsenic concentration was confirmed using 'River water' (NMIJ CRM 7202-a No.347 National Institute of Advanced Industrial Science and Technology, Japan) as a certified reference material (CRM). The average value (mean ± SD) of arsenic in the 'River water' determined in triplicate by ICP-MS analysis was 1.06 ± 0.04 µg/L (reference value, 1.18 µg/L).

2.2.4 Collection of hair and nails, and analysis of arsenic

Arsenic levels in hair and nails have been reported to provide the integrated measures for arsenic exposure (Agahian et al., 1990; Gault et al., 2008). Hair and nails of the study subjects were collected as described previously (Ali et al., 2010). Hair samples with the length of about 1 cm were collected from the region of the head close to scalp behind the ear. Nails were collected from the toes of each study subjects. Both hair and nails were washed by the method described by Chen et al. (1999). Samples were immersed in 1% Triton X-100, sonicated for 20 min, and then washed five times with milli-Q water. The washed samples were allowed to dry at 60°C overnight and digested with concentrated nitric acid using a hot plate at 70°C for 15 min and 115°C for 15 min. After cooling, the samples were diluted with 1.0% nitric acid containing yttrium (10 µg/L). The concentrations of arsenic and yttrium in these samples were determined by ICP-MS. All samples were determined in triplicate and the average values were used. Accuracy of arsenic measurement was verified using "human hair" (GBW09101, Shanghai Institute of Nuclear Research Academia Sinica, China) as a CRM. The average value of arsenic in "human hair" determined in triplicate followed by ICP-MS analysis was 0.61 ± 0.12 µg/g (reference value, 0.59 µg/g).

2.2.5 Blood pressure measurement

The standard protocol for measuring blood pressure recommended by WHO was used in this study. After study subjects had rested for 20 min or longer, both systolic and diastolic blood pressures (SBP and DBP) were measured three times with a

mercury sphygmomanometer with subjects sitting. SBP and DBP were defined at the first and fifth phase Korotkoff sounds, respectively. The average of three measurements was used for the analysis. Hypertension was defined as a SBP of ≥140 mm Hg and a DBP of ≥90 mm Hg on three repeated measurements.

2.2.6 Collection of plasma

The study participants were requested to fast overnight (10-12 hours). Fasting blood samples (5-7mL) were collected in ethylenediaminetetraacetic acid (EDTA)-containing blood collection tubes from each individual by venipuncture. The blood was then placed immediately on ice and subsequently centrifuged at 1,600 × g for 15 min at 4^{o}C. The plasma supernatant was then taken and stored at- 80^{o}C.

2.2.7 Measurements of plasma CRP, ICAM-1 and VCAM-1

Plasma levels of CRP, ICAM-1 and VCAM-1 were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits for CRP and VCAM-1 (R&D Systems, Inc. Minneapolis, USA), and ICAM-1 (Invitrogen Corporation, Camarillo, USA) according to the manufacture's protocols. On completion of the assay, the observed color change was read on a microplate reader (Mikura Ltd. UK) and CRP, ICAM-1 and VCAM-1 levels were calculated by extrapolation from a standard curve. A separate standard curve was constructed for each immunoassay. All standards and samples were analyzed in duplicate and the mean values were taken.

Principle for CRP assay: CRP assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for CRP was pre-coated onto a microplate. Standards and samples were pipetted into the wells and any CRP present was bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for CRP was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells and color developed in proportion to the amount of CRP bound in the initial step. The color development was stopped by addition of acid and then the intensity of the color was measured at 450 nm on a microplate reader. The intra and inter assay coefficients of variations (CVs) were <10%.

Principle for ICAM-1 assay: An anti-ICAM-1 monoclonal coating antibody was adsorbed onto microwells. ICAM-1 present in the sample or standard were bound to antibodies adsorbed to the microwells; an HRP-conjugated monoclonal anti-ICAM-1

antibody was added and bound to ICAM-1 captured by the first antibody. Following incubation, unbound enzyme conjugated anti-ICAM-1 was removed during a wash step and substrate solution reactive with HRP was added to the wells. A colored product was formed in proportion to the amount of soluble ICAM-1 present in the sample. The reaction was terminated by addition of acid and the absorbance was measured at 450 nm. A standard curve was prepared from five ICAM-1 standard dilutions and ICAM-1 sample concentration was determined. The intra and inter assay coefficients of variations (CVs) were <10%.

Principle for VCAM-1 assay: VCAM-1 assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for VCAM-1 was pre-coated onto a microplate. Standards, samples, controls, and conjugate were pipetted into the wells and any VCAM-1 present was sandwiched by the immobilized antibody and the enzyme-linked monoclonal antibody specific for VCAM-1. Following a wash to remove any unbound substances, a substrate solution was added to the wells and color developed in proportion to the amount of VCAM-1 bound. The color development was stopped and the intensity of the color was then measured at 450 nm. The intra and inter assay coefficients of variations (CVs) were <10%.

2.2.8 Statistical analysis

Statistical analysis for this study was performed using software of Statistical Packages for Social Sciences (SPSS version 17.0, SPSS Inc., Chicago, IL). Statistical analyses were performed after log transformation of the data due to the skewed distribution of the raw data. The differences in descriptive characteristics and the profiles of plasma circulating molecules between the study subjects of arsenic-endemic and non-endemic areas were analyzed by Independent Sample T-test and Chi-square test. Spearman correlation coefficient tests were used to evaluate the correlations between the circulating molecules and arsenic exposure metrics (water, hair and nail arsenic). Multivariate linear regression analyses were performed to assess the associations of arsenic exposure metrics with circulating molecules before and after adjusting for age, sex, BMI, smoking, hypertension, occupation, education and monthly income. To test the dose-response relationship, the study subjects of arsenic-endemic areas were split into tertile (low, medium and high) groups based on the three concentrations of arsenic in water, hair and nails. Non-endemic subjects

were used as one group for the comparison. The dose-response relationships were analyzed by One-way ANOVA followed by Bonferroni multiple comparison test. A value of $p< 0.05$ was considered statistically significant.

2.3 Results

2.3.1 Descriptive characteristics of the study participants

Table 2.1 shows the characteristics of the study subjects in the arsenic-endemic (n=218) and non-endemic areas (n=106). Arsenic concentrations in drinking water, hair and nails of the arsenic-endemic subjects were approximately 75, 17 and 7.5 times higher, respectively, than those in non-endemic subjects. Since attempts were made to match age, sex and socioeconomic parameters (occupation, monthly income and education) between arsenic-endemic and non-endemic study subjects, no significant differences were observed in those parameters between the two study groups. Most of the male study subjects were farmers and the females were housewives in both areas. Hypertensive patients were significantly higher in arsenic-endemic areas than in the non-endemic area. Higher percentage of male study subjects were non-smoker. No female was found to be a smoker. None of the study subjects drank alcohol. The average level of BMI of the study subjects in arsenic-endemic areas was slightly lower than that in non-endemic area. The average levels of diastolic blood pressure (DBP) and systolic blood pressure (SBP) in arsenic-endemic study subjects were significantly higher than that in non-endemic subjects.

Table 2.1 Descriptive characteristics of the study subjects in arsenic-endemic and non-endemic areas

Parameters	Non-endemic	Arsenic-endemic	*p*-value
Total subjects (n)	106	218	
Sex (n)			
Male	54	118	
Female	52	100	
Age	35.04 ± 10.93	37.55 ± 11.71	0.059*
Duration of residence (years)	28.43 ± 12.76	31.35 ± 13.95	0.06*
As concentration in drinking water (µg/L)	2.30 ± 2.77	173.46 ± 156.59	<0.001*
As concentration in hair (µg/g)	0.33 ± 0.25	5.63 ± 6.41	<0.001*
As concentration in nail (µg/g)	1.25 ± 1.32	9.27 ± 6.79	<0.001*
SBP (mmHg)	110.47 ± 14.43	120.76 ± 17.45	<0.001*
DBP (mmHg)	70.10 ± 9.51	78.65 ± 10.82	<0.001*
Occupation [n, (%)]			
Male			
Farmers	44 (81.48)	99 (83.9)	0.242†
Business	1 (1.9)	3 (2.5)	
Students	4 (7.4)	4 (3.4)	
Tailors	1 (1.9)	3 (2.5)	
¶Others	4 (7.6)	9 (7.5)	
Female			
Housewives	47 (90.4)	91 (91)	
Farm workers	2 (3.8)	3 (3)	
Students	0	3 (3)	
‡Others	3 (5.8)	3 (3)	
Education [n, (%)]			
No formal education	60 (56.6)	118 (54.1)	0.504†
Primary	40 (37.7)	77 (35.3)	
Secondary	5 (4.7)	21 (9.6)	
Higher	1 (0.9)	2 (0.9)	
Income/month (US$)	23.21 ± 5.46	23.96 ± 8.37	0.332*
Hypertension [n, (%)]			
Yes	2 (1.9)	30 (13.8)	< 0.01†
No	104 (98.1)	188 (86.2)	
Smoking in male [n, (%)]			
Yes	21 (38.89)	44 (37.29)	0.841†
No	33 (61.11)	74 (62.71)	
BMI (kg/m^2)	21.20 ± 2.75	20.5 ± 3.11	<0.05*

Abbreviation: As, Arsenic. Data were presented as mean ± SD. * *p*- and †*p*- values were from the Independent Sample T-test and Chi-square test, respectively. ¶Others included village doctor, carpenter, rickshaw puller, security guard and retired worker. ‡Others included farmer and labourer.

2.3.2 Comparisons of the levels of plasma circulating molecules related to atherosclerosis between arsenic-endemic and non-endemic areas

Figure 2.2 shows the plasma levels of CRP, ICAM-1 and VCAM-1 in arsenic-endemic and non-endemic study subjects. Plasma CRP levels were significantly (p<0.001) higher in arsenic-endemic subjects than those of non-endemic subjects. Similarly, ICAM-1 and VCAM-1 levels were also found to be significantly (p<0.001) higher in arsenic-endemic group than in non-endemic group.

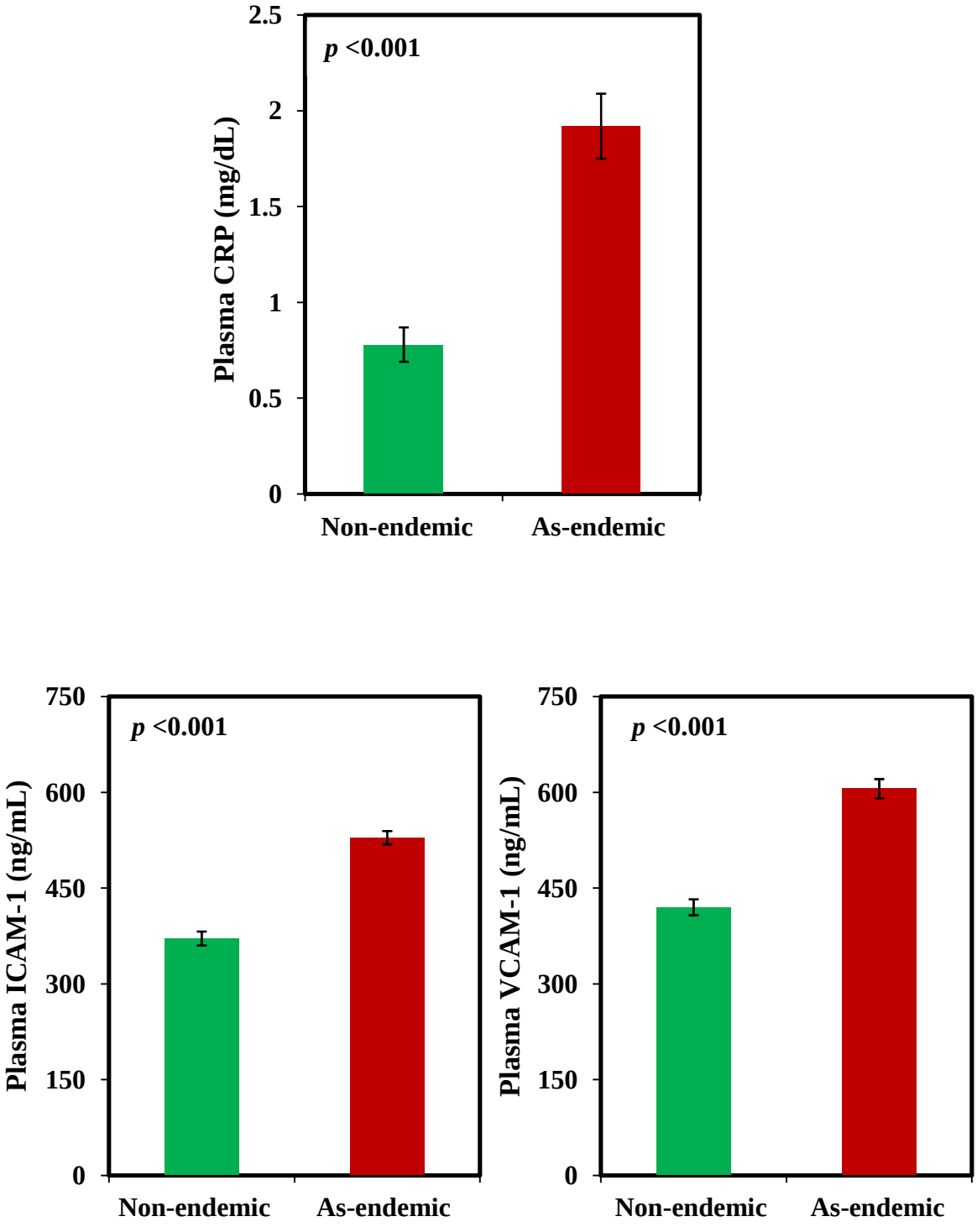

Figure 2.2 Levels of plasma CRP, ICAM-1 and VCAM-1 in the study subjects from arsenic-endemic and non-endemic areas. Data were presented as mean ± SD. Differences were analyzed by Independent Sample T-test.

2.3.3 Correlations of arsenic exposure metrics with plasma circulating molecules

Figure 2.3A, 2.3B and 2.3C show the correlations between arsenic exposure metrics and plasma CRP, ICAM-1 and VCAM-1 molecules involved in atherosclerosis. Plasma CRP levels were significantly increased with increasing concentrations of arsenic in water (r_s= 0.354, p<0.001), hair (r_s= 0.339, p<0.001) and nails (r_s= 0.227, p<0.001) (Figure 2.3A). Similar positive correlations were also observed in case of plasma ICAM-1 (r_s= 0.371, p<0.001 for water; r_s= 0.376, p<0.001 for hair and r_s= 0.334, p<0.001 for nail) and VCAM-1 (r_s= 0.313, p<0.001 for water; r_s= 0.372, p<0.001 for hair and r_s= 0.300, p<0.001 for nail) levels with water, hair and nail arsenic concentrations (Figure 2.3B and 2.3C).

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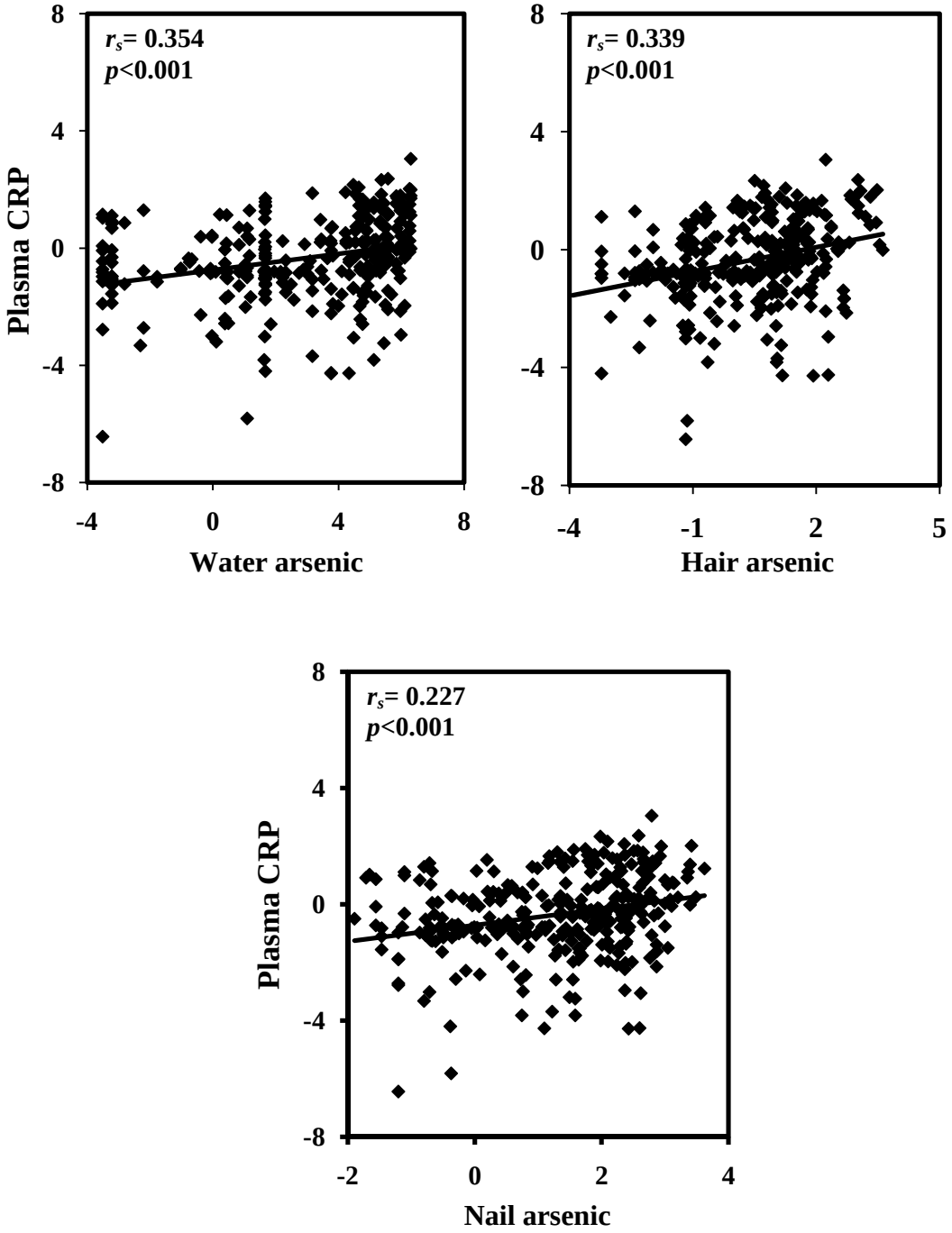

Figure 2.3A Correlation of arsenic exposure with plasma CRP levels. Log-transformed values of CRP, water, hair and nail arsenic were used. r_s and *p*-values were from Spearman correlations coefficient test.

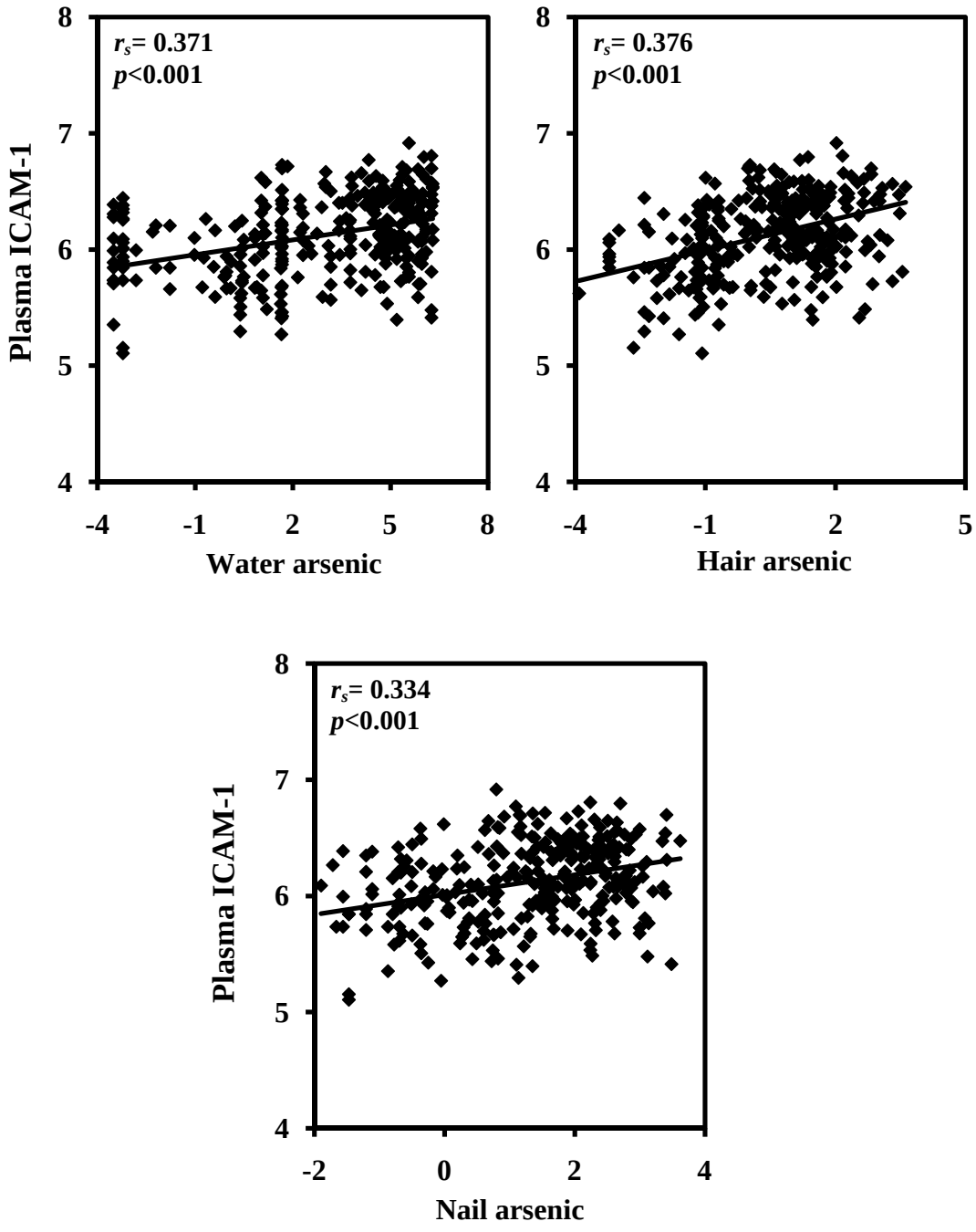

Figure 2.3B Correlation of arsenic exposure with plasma ICAM-1 levels. Log-transformed values of ICAM-1, water, hair and nail arsenic were used. r_s and *p*-values were from Spearman correlations coefficient test**.**

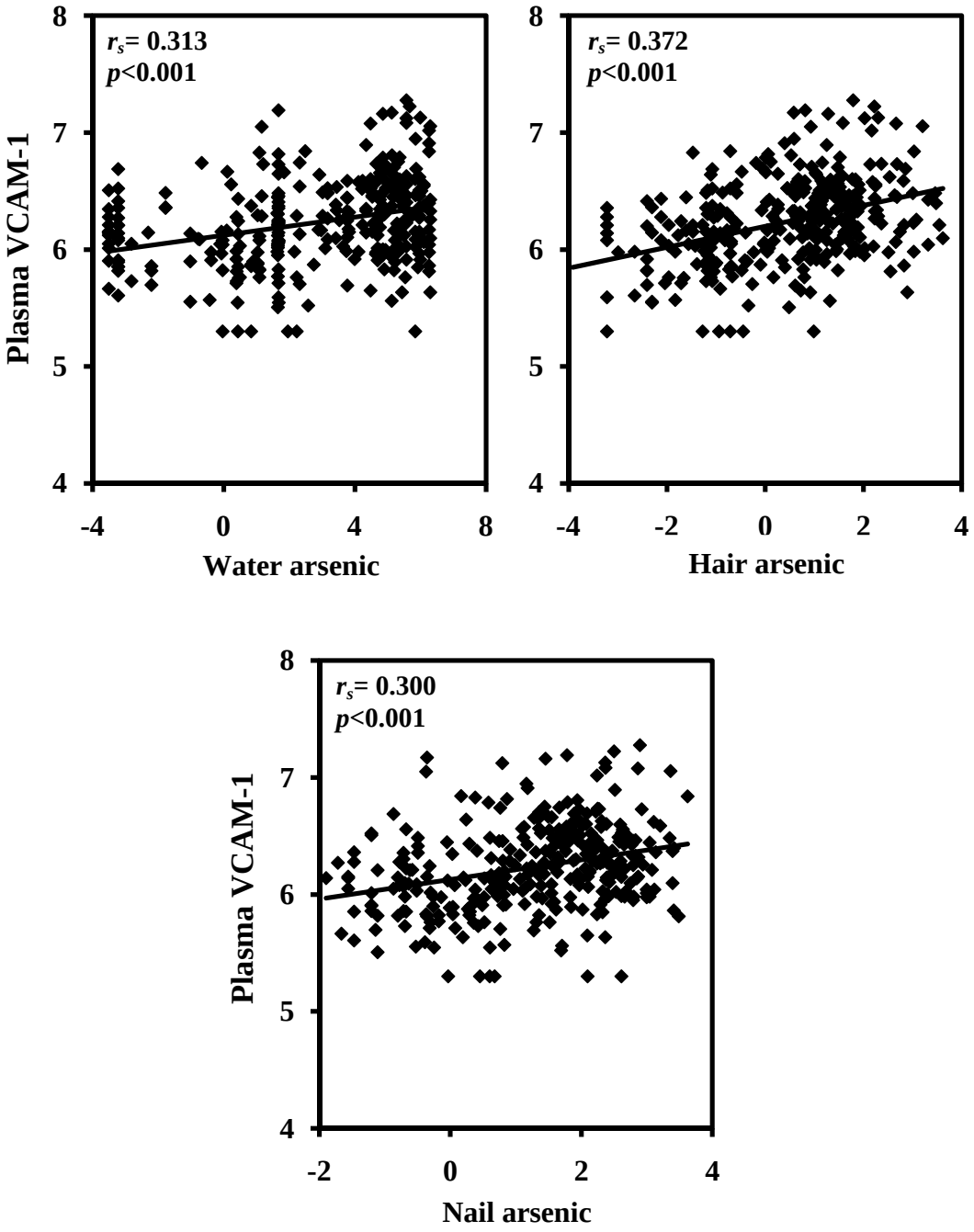

Figure 2.3C Correlation of arsenic exposure with plasma VCAM-1 levels. Log-transformed values of VCAM-1, water, hair and nail arsenic were used. r_s and *p*-values were from Spearman correlations coefficient test**.**

2.3.4 Association between arsenic exposure metrics and plasma circulating molecules through multivariate linear regression analysis

Multivariate linear regression analyses were performed to evaluate the effect of age, sex, BMI, smoking, hypertension, occupation, education and monthly income as covariates on the associations of plasma circulating molecules with arsenic exposure metrics (water, hair and nails). All the associations of plasma circulating molecules with arsenic exposure metrics were statistically significant even after adjusting for those covariates (Table 2.2).

Table 2.2 Association between arsenic exposure metrics and plasma circulating molecules in individuals through multivariate linear regression analysis

Dependent variable	Independent variable					
	Water arsenic IQR[a]		Hair arsenic IQR[b]		Nail arsenic IQR[c]	
	Before adjustment	After adjustment	Before adjustment	After adjustment	Before adjustment	After adjustment
CRP						
β-Coefficient	0.136	0.139	0.27	0.276	0.275	0.269
(95% CI)	(0.085 - 0.188)	(0.084 - 0.193)	(0.175 - 0.364)	(0.177 - 0.374)	(0.158 - 0.392)	(0.147 - 0.391)
Change by IQR[d]	0.709	0.724	0.394	0.403	0.596	0.583
(95% CI)	(0.658 - 0.759)	(0.669, 0.779)	(0.3 - 0.489)	(0.305 - 0.501)	(0.481 - 0.712)	(0.462 - 0.705)
ICAM-1						
β-Coefficient	0.045	0.042	0.096	0.091	0.092	0.087
(95% CI)	(0.033 - 0.058)	(0.029 - 0.055)	(0.073 - 0.119)	(0.068 - 0.114)	(0.063 - 0.121)	(0.058 - 0.116)
Change by IQR[d]	0.234	0.219	0.14	0.133	0.199	0.187
(95% CI)	(0.221 - 0.248)	(0.205 - 0.233)	(0.117 - 0.164)	(0.109, 0.156)	(0.170 - 0.229)	(0.159 - 0.218)
VCAM-1						
β-Coefficient	0.039	0.036	0.09	0.086	0.086	0.081
(95% CI)	(0.025 - 0.052)	(0.023 - 0.05)	(0.066 - 0.114)	(0.062 - 0.110)	(0.056 - 0.116)	(0.051 - 0.111)
Change by IQR[d]	0.203	0.188	0.131	0.125	0.186	0.176
(95% CI)	(0.189 - 0.217)	(0.174 - 0.201)	(0.108 - 0.155)	(0.102 - 0.149)	(0.157 - 0.216)	(0.146 - 0.205)

Abbreviation: IQR, Inter-quartile range. Log-transformed values of arsenic exposure metrics and plasma circulating molecules were used. Age, sex, BMI, smoking, hypertension, occupation, education and monthly income were adjusted as covariates. [a]Water IQR (2.98, 186), [b]hair IQR (0.393, 4.703) and [c]nail IQR (1.430, 10.173). [d]Change in outcome associated with an IQR increase in arsenic.

2.3.5 Dose-response relationship of arsenic exposure with circulating molecules

In Table 2.3, dose-response relationships were examined in non-endemic and tertile (low, medium and high) groups of arsenic-endemic subjects. Results showed that there were some general trends in changing ICAM-1 and VCAM-1 in the low, medium and high arsenic exposure groups. ICAM-1 and VCAM-1 were significantly changed in the higher (low, medium and high) exposure groups compared to the non-endemic group. Among the plasma biomarkers CRP showed relatively good shape of dose-response relationships with arsenic exposure. CRP levels were also gradually increased in the higher exposure gradients except the medium group of hair arsenic concentrations. CRP levels in the endemic tertile groups of water, hair and nail arsenic concentrations were also significantly different from the non-endemic group with the exception of the low groups of water and nail arsenic concentrations. Moreover, CRP levels were also significantly different for medium versus low, high versus medium and high versus low group of water arsenic concentrations.

Table 2.3 Dose-response relationships between arsenic exposure levels in water, hair, and nails, and the levels of plasma circulating molecules

Dependent variable	Independent variable	Non-endemic	Low	Medium	High	*p*-value (F-test)
CRP (mg/L)	Water As	0.78 ± 0.88	1.15 ± 1.53	1.75 ± 1.96 [a,b]	2.82 ± 3.28 [a,b,c]	<0.001
	Hair As		1.74 ± 2.22 [a]	1.37 ± 1.59 [a]	2.64 ± 3.19 [a]	<0.001
	Nail As		1.39 ± 1.68	1.99 ± 2.29 [a]	2.33 ± 3.14 [a]	<0.001
ICAM-1 (ng/mL)	Water As	371.4 ± 112.2	518.1 ± 155.2 [a]	520.3 ± 135.0 [a]	549.2 ± 170.3 [a]	<0.001
	Hair As		548.9 ± 160.1 [a]	519.3 ± 137.2 [a]	520.5 ± 164.5 [a]	<0.001
	Nail As		530.9 ± 177.8 [a]	533.5 ± 143.2 [a]	523.8 ± 141.5 [a]	<0.001
VCAM-1 (ng/mL)	Water As	420.3 ± 129.9	589.7 ± 184.8 [a]	604.1 ± 216.3 [a]	623.7 ± 261.1 [a]	<0.001
	Hair As		605.1 ± 220.1 [a]	588.4 ± 201.3 [a]	624.2 ± 245.7 [a]	<0.001
	Nail As		602.1 ± 237.6 [a]	627.3 ± 200.1 [a]	589.1 ± 229.9 [a]	<0.001

Abbreviation: As, Arsenic. Data were presented as mean ± SD. Statistically significant association between exposure level and the levels of circulating molecules in One-Way ANOVA was examined by F-test, followed by Bonferroni multicomparison test between each group of exposure level. [a] Significantly difference from non-endemic group; [b] Significantly different from 'low' group. [c] Significantly different from 'medium' group. As levels in water; non-endemic (0.03 – 13.17 µg/L; n = 106), low (0.46-69.4 µg/L; n = 72), medium (76-205 µg/L; n = 72), and high (214-546 µg/L; n = 74). As levels in hair; non-endemic (0.03-1.62 µg/g; n = 106), low (0.25-2.37 µg/g; n = 71), medium (2.45-4.95 µg/g; n = 73), and high (5-37.24 µg/g; n = 74). As levels in nail; non-endemic (0.15-8.13 µg/g; n = 106), low (0.53-5.14 µg/g; n = 72), medium (5.21-10.65 µg/g; n = 72), and high (10.67-37.42; n = 72).

2.4 Discussion

Although it has been well established that chronic exposure to arsenic is associated with CVDs, uncertainties remain in the etiology of arsenic-induced CVDs. In the present study, we demonstrated the characteristic changes in biochemical indicators for atherosclerosis; high levels of CRP, ICAM-1 and VCAM-1 among the residents in arsenic-endemic areas in Bangladesh as compared to the non-endemic residents. Arsenic exposure (water, hair and nails arsenic) showed significant associations with those biochemical indicators (Figure 2.3A, 2.3B and 2.3C). Further, in the dose-response relationship, although there were some general trends in changing plasma biomarkers in the higher exposure gradients compared to the lower exposure gradients but the significant changes of the plasma markers were largely

limited to non-endemic versus each tertile of endemic subjects. Among these circulating molecules CRP showed relatively good shape of dose-response relationships in arsenic-endemic tertile groups (Table 2.3).

Mounting evidence has suggested that arsenic exposure increases the risk of vascular diseases (Chen et al., 2011; Tseng et al., 2003). Development of atherosclerosis is the fundamental step of CVDs. Preclinical manifestations of atherosclerosis as examined by carotid artery plaques and thickness have been observed in the residents exposed to arsenic both in Taiwan and in Bangladesh (Chen et al., 2006; Wang et al., 2009). However, very few studies have reported the changes in biochemical indicators for atherosclerosis in human exposed to arsenic (Chen et al., 2007; Wu et al., 2012). Recently, the significance of biochemical indicators for pro-oxidative and pro-inflammatory lesions such as oxidized low-density lipoprotein (Ox-LDL), ICAM-1, VCAM-1, and CRP rather than traditional lipid markers such as total cholesterol and LDL has been highlighted for assessing the development of atherosclerosis.

ICAM-1 and VCAM-1 expressed in endothelial cells play important roles in the recruitment and trans-endothelial migration of leukocytes, leading to the initiation of atherosclerosis (Galkina and Ley, 2007). Reactive oxygen species, pro-inflammatory cytokines, CRP, and Ox-LDL have been shown to induce the expression of ICAM-1 and VCAM-1 in the endothelial cells, resulting in the elevation of soluble forms of ICAM-1 and VCAM-1 in blood plasma (Cook-Mills et al., 2011; Zhanget al., 2012). The studies conducted previously in the different areas of Bangladesh showed slight but significant increases in soluble ICAM-1 and VCAM-1 levels in plasma of arsenic-endemic residents (Chen et al., 2007; Wu et al., 2012). In the present study, however, plasma ICAM-1 and VCAM-1 levels were increased in concert with those of CRP among arsenic-endemic individuals. These results provide much clearer insight into the interaction of pro-inflammatory events and adhesion molecules induced by arsenic exposure.

Emerging evidence has suggested an important role of CRP both as a powerful predictor of CVDs and as a player in the development of atherosclerosis (Blake et al., 2003). The circulating CRP as well as endogenously produced CRP is known to induce the release of pro-inflammatory cytokines from monocytes (Ballou and Lozanski, 1992) and promote the expression of ICAM-1 and VCAM-1 in endothelial

cells (Wadhamet al., 2004). Furthermore, CRP accelerates the monocyte adhesion to endothelial cells and the uptake of Ox-LDL by endothelial cells via the activation of LOX-1, which is a receptor for Ox-LDL (Li et al., 2004). Thus, the increases in plasma levels of ICAM-1 and VCAM-1 concomitantly with CRP in arsenic-endemic residents suggest that arsenic-induced inflammatory events are involved in pathological activation of ICAM-1 and VCAM-1 in the endothelial cells. CVDs are major cause of mortality worldwide. Even a small increased risk associated with arsenic exposure can cause a large number of excess deaths. Therefore, arsenic exposure related CVDs could be of a public health concern in countries whose population are exposed to elevated concentration of arsenic.

The major strengths of this study were: 1) wide range of arsenic concentrations in drinking water, hair and nails of the study subjects that provided strong dose-response relationship between arsenic exposure and biochemical markers for atherosclerosis 2) all associations were shown using three ways of exposure metrics (water, hair and nail arsenic). Drinking water arsenic is recognized as external exposure metric, whereas hair and nail arsenic are recognized as internal exposure metrics. Arsenic levels in nail and hair samples have been reported to provide the integrated measure for arsenic exposure (Agahian et al., 1990; Karagas et al., 1996). One centimeter of hair reflects approximately one month of exposure. On the other hand, nail capture historical exposure to arsenic from several months to a year. Therefore, correlations of biochemical markers for atherosclerosis with these three exposure metrics reduced the possibilities of misclassification of exposure and effects of other confounders on the observed associations. Although this study represented an extensive epidemiological research, the present study should be interpreted with cautions. First, we adjusted age, sex, BMI, smoking, hypertension, occupation, education and monthly income as covariates (Table 2.2) but there may be some other additional factors that might influence the results. Second, this study was designed to be cross sectional, but not prospective. A cohort based study is required in future for precise cause effect relationship between circulating molecules related to atherosclerosis and arsenic exposure. Third, the most of the study subjects had poor socioeconomic conditions and their BMI were in the lower end of normal range of BMI. Thus, the results of the current study may not be generalizable to other study populations because of the different distribution of risk factors for atherosclerosis that may influence the effect of

arsenic exposure. Nevertheless, this study suggests that exposure to arsenic causes pro-inflammatory conditions that may lead to the development of atherosclerosis.

2.5 Conclusion

The present study has demonstrated that circulating levels of plasma CRP, ICAM-1 and VCAM-1 were significantly higher in arsenic-endemic subjects than those in the non-endemic subjects. Further, CRP, ICAM-1, and VCAM-1 showed dose-response relationships with arsenic exposure metrics. The associations of these biochemical indicators with arsenic exposure metrics were significant even after adjusting for relevant covariates. Therefore, all the associations observed in this study may be the hallmark features of arsenic-induced pro-inflammatory events leading to atherosclerosis. The biochemical indicators measured in this study strongly suggest that the residents in arsenic-endemic areas are at risk for atherosclerosis and future development of CVDs.

2.6 References

Agahian, B., Lee, J. S., Nelson, J. H., and Johns, R. E. (1990). Arsenic levels in fingernails as a biological indicator of exposure to arsenic. *Am. Ind. Hyg. Assoc. J.* **51**, 646-651.

Ali, N., Hoque, M. A., Haque, A., Salam, K. A., Karim, M. R., Rahman, A., Islam, K., Saud, Z. A., Khalek, M. A., Akhand, A. A., Hossain, M., Mandal, A., Karim, M. R., Miyataka, H., Himeno, S., and Hossain, K. (2010). Association between arsenics exposure and plasma cholinesterase activity: a population based study in Bangladesh. *Environ. Health* **9**, 36.

Ballou, S. P., and Lozanski, G. (1992). Induction of inflammatory cytokine release from cultured human monocytes by C-reactive protein. *Cytokine* **4**, 361-368.

BGS. (2001). Arsenic contamination of ground water in Bangladesh. http://www.bgs.ac.uk/research/groundwater/health/arsenic/Bangladesh.

Blake, G. J., Rifai, N., Buring, J. E., and Ridker, P. M. (2003). Blood pressure, C-reactive protein, and risk of future cardiovascular events. *Circulation* **108**, 2993-2999.

Black, S., Kushner, I., and Samols, D. (2004). C-reactive protein. *J. Biol. Chem*. **279**, 48487-48490.

Blankenberg, S., Rupprecht, H. J., Bickel, C., Peetz, D., Hafner, G., Tiret, L., and Meyer, J. (2001). Circulating cell adhesion molecules and death in patients with coronary artery disease. *Circulation* **104**, 1336-1342.

Calabro, P., Willerson, J. T., and Yeh, E. T. (2003). Inflammatory cytokines stimulated C-reactive protein production by human coronary artery smooth muscle cells. *Circulation* **108**, 1930-1932.

Caldwell, B. K., Caldwell, J. C., Mitra, S. N., and Smith, W. (2003). Tubewells and arsenic in Bangladesh: challenging a public health success story. *Int. J. Popul. Geogr*. **9**, 23-38.

Castell, J. V., Gomez-Lechon, M. J., David, M., Fabra, R., Trullenque, R., and Heinrich, P. C. (1990). Acute-phase response of human hepatocytes: regulation of acute-phase protein synthesis by interleukin-6. *Hepatology* **12**, 1179-1186.

Chen, K. L., Amarasiriwardena, C. J., and Christiani, D. C. (1999). Determination of total arsenic concentrations in nails by inductively coupled plasma mass spectrometry. *Biol. Trace Elem. Res.* **67**, 109-125.

Chen, Y., Graziano, J. H., Parvez, F., Liu, M., Slavkovich, V., Kalra, T., Argos, M., Islam, T., Ahmed, A., Rakibuz-Zaman, M., Hasan, R., Sarwar, G., Levy, D., van Geen, A., and Ahsan, H. (2011). Arsenic exposure from drinking water and mortality from cardiovascular disease in Bangladesh: prospective cohort study. *BMJ* **362**, d2431.

Chen, Y., Hakim, M. E., Parvez, F., Islam, T., Rahman, A. M., and Ahsan, H. (2006). Arsenic exposure from drinking-water and carotid artery intima-medial thickness in healthy young adults in Bangladesh. *J. Health Popul. Nutr.* **24**, 253-257.

Chen, Y., Santella,R. M., Kibriya, M. G., Wang, Q., Kappil, M., Verret, W. J., Graziano, J. H., and Ahsan, H. (2007). Association between arsenic exposure from drinking water and plasma levels of soluble cell adhesion molecules. *Environ. Health Perspect*. **115**, 1415-1420.

Cheng, T. J., Chuu, J. J., Chang, C. Y., Tsai, W. C., Chen, K. C., and Guo, H. R. (2011). Atherosclerosis induced by arsenic in drinking water in rats through altering lipid metabolism. *Toxicol. Appl. Pharmacol.* **256**, 146-153.

Chowdhury, A. M. R., (2004). Arsenic crisis in Bangladesh. *Sci. Am*. **291**, 86-91.

Chowdhury, U. K., Biswas, B. K., Chowdhury, T. R., Samanta, G., Mandal, B. K., Basu, G. C., Chanda, C. R., Lodh, D., Saha, K. C., Mukherjee, S. K., Roy, S., Kabir, S., Quamruzzaman, Q., and Chakraborti, D. (2000). Groundwater arsenic contamination in Bangladesh and West Bengal, India. *Environ. Health Perspect.* **108**, 393-397.

Cook-Mills, J. M., Marchese, M. E., and Abdala-Valencia, H. (2011). Vascular cell adhesion molecule-1 expression and signaling during disease: regulation by reactive oxygen species and antioxidants. *Antioxid. Redox Signal.* **15**, 1607-1638.

Druwe, I. L, Sollome, J. J, Sanchez-Soria, P., Hardwick, R. N., Camenisch, T. D., and Vaillancour, R. R. (2012). Arsenite activates NFκB through induction of C-reactive protein. *Toxicol. Appl. Pharmacol*. **261**, 263-270.

Gahmberg, C. G., Tolvanen, M. and Kotovuori, P. (1997). Leukocyte adhesion. *Eur. J. Biochem.* **245**, 215-232.

Galkina, E., and Ley, K. (2007). Vascular adhesion molecules in atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* **27**, 2292-2301.

Gault, A. G., Rowland, H. A., Charnock, J. M., Wogelius, R. A., Gomez-Morilla, I., Vong, S., Leng, M., Samreth, S., Sampson, M. L., and Polya, D. A. (2008). Arsenic in hair and nails of individuals exposed to arsenic-rich ground waters in Kandal province, Cambodia. *Sci. Total Environ.* **393**, 168-176.

Haverkate, F., Thompson, S. G., Pyke, S. D., Gallimore, J. R., and Pepys, M. B. (1997). Production of C-reactive protein and risk of coronary events in stable and unstable angina. European Concerted Action on Thrombosis and Disabilities Angina Pectoris Study Group. *Lancet* **349**, 462-466.

Hirschfield, G. M., and Pepys, M. B. (2003). C-reactive protein and cardiovascular disease: new insights from an old molecule. *QJM* **96**, 793-807.

Hope, S. A., and Meredith, I. T. (2003). Cellular adhesion molecules and cardiovascular disease. Part II. Their association with conventional and emerging risk factors, acute coronary events and cardiovascular risk prediction. *Intern. Med. J.* **33**, 450-462.

Hossain, E., Islam, K., Yeasmin, F., Karim, M. R., Rahman, M., Agarwal, S., Hossain, S., Aziz, A., Mamun, A. A., Sheikh, A., Haque, A., Hossain, M. T., Hossain, M., Haris, P. I., Ikemura, N., Inoue, K., Miyataka, H., Himeno, S., and Hossain, K. (2012). Elevated levels of plasma Big endothelin-1 and its relation to hypertension and skin lesions in individuals exposed to arsenic. *Toxicol. Appl. Pharmacol.* **259**, 187-194.

Hwang, S. J., Ballantyne, C. M., Sharrett, A. R., Smith, L. C., Davis, C. E., Gotto, A. M. Jr., and Boerwinkle, E. (1997). Circulating adhesion molecules VCAM-1, ICAM-1, and E-selectin in carotid atherosclerosis and incident coronary heart disease cases: the Atherosclerosis Risk In Communities (ARIC) study. *Circulation* **96**, 4219-4225.

Karagas, M. R., Morris, J. S., Weiss, J. E., Spate, V., Baskett, C., Greenberg, E. R. (1996). Toenail samples as an indicator of drinking water arsenic exposure. *Cancer. Epidemiol. Biomarkers Prev.* **5**, 849-852.

Karim, M. R., Haque, A., Islam, K., Ali, N., Salam, K.A., Saud, Z. A., Hossain, E., Fajol, A., Akhand, A. A., Himeno, S., and Hossain, K. (2010). Interaction between chronic arsenic exposure via drinking water and plasma lactate dehydrogenase activity. *Sci. Total Environ.* **409**, 278-283.

Kligerman, A. D., Doerr, C. L., Tennant, A. H., Harrington-Brock, K., Allen, J. W., Winkfield, E., Poorman-Allen, P., Kundu, B., Funasaka, K., Roop, B. C., Mass, M. J., and DeMarini, D. M. (2003). Methylated trivalent arsenicals as candidate ultimate genotoxic forms of arsenic: induction of chromosomal mutations but not gene mutations. *Environ. Mol. Mutagen.* **42**, 192-205.

Lagrand, W. K., Nijmeijer, R., Niessen, H. W. M., Visser, C. A., Hermens W. Th., and Hack, C. E. (2002). C-reactive protein as a pro-inflammatory mediator in cardiovascular disease by its ability to activate complement: additional proof and hypothetical mechanisms. *Neth. Heart J.* **10**, 189-197.

Li, L., Roumeliotis, N., Sawamura, T., and Renier, G. (2004). C-reactive protein enhances LOX-1 expression in human aortic endothelial cells: relevance of LOX-1 to C-reactive protein-induced endothelial dysfunction. *Circ. Res.* **95**, 877-883.

Libby, P. (2002). Inflammation in atherosclerosis. *Nature* **420**, 868-874.

Libby, P., and Ridker, P. M. (2004). Inflammation and atherosclerosis: role of C-reactive protein in risk assessment. *Am. J. Med.* **116**, 9S-16S.

Madjid, M., Casscells, S., and Willerson, J. T. (2007). Inflammatory biomarkers as surrogate markers in detection of vulnerable plaques and vulnerable patients. In: Willerson, J. T (ed). Cardiovascular Medicine, 3rd edn.Springer, London, UK. PP 641-651.

Mulvihill, N. T., Foley, J. B., Crean, P., and Walsh, M. (2002). Prediction of cardiovascular risk using soluble cell adhesion molecules. *Eur. Heart J.* **23**, 1569-1574.

Navas-Acien, A., Sharrett, A. R., Silbergeld, E. K., Schwartz, B. S., Nachman, K. E., Burke, T. A., and Guallar, E. (2005). Arsenic exposure and cardiovascular disease: a systematic review of the epidemiologic evidence. *Am. J. Epidemiol.* **162**, 1037-1049.

Paffen, E., and deMaat, M. P. M. (2006). C-reactive protein in atherosclerosis: A causal factor? *Cardiovas. Res.* **71**, 30-39.

Pepys, M. B., and Hirschfield, G. M. (2001). C-reactive protein and atherothrombosis. *Ital. Heart. J.* **2**, 196-199.

Rahman, M., Tondel, M., Ahmad, S. A., Chowdhury, I. A., Faruquee, M. H., and Axelson, O. (1999). Hypertension and arsenic exposure in Bangladesh. *Hypertension* **33**, 74-78.

Ridker, P. M., Glynn, R. J., and Hennekens, C. H. (1998). C-reactive protein adds to the predictive value of total and HDL cholesterol in determining risk of first myocardial infarction. *Circulation* **97**, 2007-2011.

Ridker, P. M., Hennekens, C. H., Buring, J. E., and Rifari, N. (2000). C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N. Engl. J. Med*. **342**, 836-843.

Rohde, L. E., Lee, R. T., Rivero, J., Jamacochian, M., Arroyo, L. H., Briggs, W., Rifai, N., Libby, P., Creager, M. A., and Ridker, P. M. (1998). Circulating cell adhesion molecules are correlated with ultrasound-based assessment of carotid atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* **18**, 1765-1770.

Ross, R. (1999). Atherosclerosis is an inflammatory disease. *Am. Heart J.* **138**, 419-420.

Schmidt, C., Hulthe, J., and Fagerberg, B. (2009). Baseline ICAM-1 and VCAM-1 are increased in initially healthy middle-aged men who develop cardiovascular disease during 6.6 years of follow-up. *Angiology* **60**, 108-114.

Sesso, H. D., Buring, J. E., Rifai, N., Blake, G. J., Gaziano, J. M., and Ridker, P. M. (2003). C-reactive protein and the risk of developing hypertension. *JAMA* **290**, 2945-2951.

Simundic, A. M., Basic, V., Topic, E., Demarin, V., Vrkic, N., Kunovic, B., Stefanovic, M., and Begonja, A. (2004). Soluble adhesion molecules in acute ischemic stroke. *Clin. Invest. Med*. **27**, 86-92.

Springer, T. A. (1994). Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell* **76**, 301-314.

Steinberg, D. (2002). Atherogenesis in perspective: hypercholesterolemia and inflammation partners in crime. *Nat. Med.* **8**, 1211-1217.

Tilg, H., Nordberg, J., Vogel, W., Luger, T. A., Herold, M., Aulitzky, W. E., Margreiter, R., and Huber, C. (1992). Circulating serum levels of interleukin 6 and C-reactive protein after liver transplantation. *Transplantion*. **54**, 142-146.

Tseng, C. H., Chong, C. K., Tseng, C. P., Hsueh, Y. M., Chiou, H. Y., Tseng, C. C., and Chen, C. J. (2003). Long-term arsenic exposure and ischemic heart disease in arseniasis-hyperendemic villages in Taiwan. *Toxicol. Lett.* **137**, 15-21.

van Geen, A., Zheng, Y., Goodbred, S. Jr., Horneman, A., Aziz, Z., Cheng, Z., Stute, M., Mailloux, B., Weinman, B., Hoque, M. A., Seddique, A. A., Hossain, M. S., Chowdhury, S. H., and Ahmed, K. M. (2008). Flushing history as a hydrogeological control on the regional distribution of arsenic in shallow groundwater of the Bengal Basin. *Environ. Sci. Technol*. **42**, 2283-2288.

Wadham, C., Albanese, N., Roberts, J., Wang, L., Bagley, C. J., Gamble, J. R., Rye, K. A., Barter, P. J., Vadas, M. A., and Xia, P. (2004). High-density lipoproteins neutralize C-reactive protein proinflammatory activity. *Circulation* **109**, 2116-2122.

Wang, C. H., Chen, C. L., Hsiao, C. K., Chiang, F. T., Hsu, L. I., Chiou, H. Y., Hsueh, Y. M., Wu, M. M., and Chen, C. J. (2009). Increased risk of QT prolongation associated with atherosclerotic diseases in arseniasis-endemic area in southwestern coast of Taiwan. *Toxicol. Appl. Pharmacol*. **239**, 320-324.

Wang, C. H., Hsiao, C. K., Chen, C. L., Hsu, L. I., Chiou, H. Y., Chen, S. Y., Hsueh, Y. M., Wu, M. M., and Chen, C. J. (2007). A review of the epidemiologic literature on the role of environmental arsenic exposure and cardiovascular diseases. *Toxicol .Appl. Pharmacol.* **222**, 315-326.

Wang, C. H., Jeng, J. S., Yip, P. K., Chen, C. L., Hsu, L. I., Hsueh, Y. M., Chiou, H. Y., Wu, M. M., and Chen, C. J. (2002). Biological gradient between long-term arsenic exposure and carotid atherosclerosis. *Circulation* **105**, 1804-1809.

Wu, F., Jasmine, F., Kibriya, M. G., Liu, M., Wójcik, O., Parvez, F., Rahaman, R., Roy, S., Paul-Brutus, R., Segers, S., Slavkovich, V., Islam, T., Levy, D., Mey, J. L., van Geen, A., Graziano, J. H., Ahsan, H., and Chen, Y. (2012). Association between arsenic exposure from drinking water and plasma levels of cardiovascular markers. *Am. J. Epidemiol*. **175**, 1252-1261.

Zhang, Y. C., Wei, J. J., Wang, F., Chen, M. T., and Zhang, M. Z. (2012). Elevated levels of oxidized low-density lipoprotein correlate positively with C-reactive protein in patients with acute coronary syndrome. *Cell Biochem. Biophys.* **62**, 365-372.

Chapter 3: Exploring the association of arsenic exposure with serum vascular endothelial growth factor in arsenic-endemic individuals in Bangladesh

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3. Abstract

Arsenic is an environmental pollutant that causes a variety of health hazards affecting millions of people in the world. Arsenic exposure is associated with cancer and vascular diseases. Angiogenesis is an important step for the pathological development of cancer and vascular diseases. Vascular endothelial growth factor (VEGF) is a specific marker for angiogenesis. However, human study showing the association between arsenic exposure and serum VEGF levels has not yet been documented. This study was aimed to investigate the association between arsenic exposure and serum VEGF levels in the arsenic-endemic individuals in Bangladesh. A total of 260 individuals were recruited for this study. Arsenic exposure levels were measured by Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) and serum VEGF levels were quantified using VEGF immunoassay kit. The study subjects were stratified into tertile (low, medium and high) groups based on the arsenic concentrations in water, hair and nails. Serum VEGF levels were significantly correlated with water (r_s = 0.363, $p < 0.001$), hair (r_s = 0.205, $p < 0.01$) and nail (r_s = 0.190, $p < 0.01$) arsenic. Further, serum VEGF levels showed dose-response relationships in medium versus low ($p < 0.05$), and high versus low ($p < 0.001$) exposure groups of water arsenic and medium versus low ($p < 0.05$), and high versus low ($p < 0.01$) exposure groups of hair and nail arsenic concentrations. Dose-response relationships were also tested when the subjects were divided into three groups (≤10 μg/L, 10.1-50 μg/L and >50 μg/L) based on the regulatory upper limit of water arsenic concentrations set by WHO (10 μg/L) and Bangladesh Government (50 μg/L). Mean VEGF levels in ≤10 μg/L, 10.1-50 μg/L and >50 μg/L groups were 91.84, 129.54, and 169.86 pg/mL, respectively, however, significant ($p < 0.01$) differences in VEGF levels were only found in >50 μg/L versus ≤10 μg/L groups. Significant association of arsenic exposure with VEGF levels were found even after adjusting for relevant covariates. Therefore, these results provide evidence that arsenic exposure has a pro-angiogenic effect on humans, which may be implicated in arsenic-induced tumorigenesis and vascular diseases.

3.1 Introduction

Arsenic is a potent environmental pollutant and human carcinogen that is ubiquitously present in food, soil, water and airborne particles. Chronic arsenic poisoning has become a major public health concern in many countries. Arsenic poisoning has taken a serious turn affecting millions of people in Bangladesh (Smith et al., 2000). Many people have died of the chronic diseases caused by prolonged exposure to arsenic. It has been assumed that approximately 80 million people are at risk of arsenic poisoning in the country (Caldwell et al., 2003; Chowdhury A, 2004; Chowdhury U et al., 2000). World Health Organization (WHO) has described arsenic toxicity in Bangladesh as the largest mass poisoning of a population in history. The major forms of arsenic detected in groundwater are inorganic arsenite (As^{III}) and arsenate (As^{V}). Ingestion of inorganic arsenic through drinking water has been reported to be associated with a variety of cancers, dermatitis, cardiovascular diseases, peripheral neuropathy, diabetes mellitus, renal failure and liver dysfunction (Chen C et al., 2007; Chen Y et al., 2011; Hossain et al., 2012; Islam et al., 2011; Lai et al., 1994; Mazumder et al., 1998; Meliker et al., 2007; Tapio and Grosche 2006; Vahidnia et al., 2008).

Vascular endothelial growth factor (VEGF) is a heparin-binding homodimeric glycoprotein. It is also known as vascular permeability factor because of its ability to induce vascular leakage. VEGF plays a central role in both vasculogenesis, the formation of new blood vessels from the embryonic origin, and angiogenesis, the formation of new blood vessels from pre-existing ones. VEGF is released into the circulating system by hypoxic cells, activated platelets, leukocytes, and cancer cells (Banks et al., 1998; Gunsilius et al., 2000; Mohle et al., 1997; Verheul et al., 1997; Webb et al., 1998). VEGF family consist of VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E and placental growth factor, each of which is transcribed by individual genes (Ferrara 2002; Neufield et al., 1999). VEGF-A is generally called VEGF because of its key role in vasculogenesis, angiogenesis and differentiation of progenitor endothelial cells (Takahashi et al., 2011). VEGF–A exists in at least four different isoforms of 206, 189, 165, and 121 amino acids and they are generated by alternative splicing of mRNA (Houck et al., 1991; Leung et al., 1989; Tischer et al., 1991). It appears that $VEGF_{121}$ and $VEGF_{165}$ are secreted as a soluble form, whereas other two isoforms remain as cell-associated forms. All VEGF-A isoforms apparently show

identical biological activity, but $VEGF_{165}$ is dominant form because of its larger amount and greater extent of biological activity. $VEGF_{165}$ has been implicated in pathological angiogenesis associated with tumor, neovascular diseases and other conditions. Because of its specific role in tumorigenesis and cancer development, VEGF-mediated signals have been targeted for the therapeutic intervention of cancer (Shinkaruk et al., 2003).

VEGF expression have been observed in a variety of cell types during atherosclerosis, myocardial ischemia, diabetic and ischemic retinopathy, tumorigenesis, arthritis, psoriasis and wound healing (Carmeliet and Collen, 2000). The biological effects of VEGF are mediated by binding with its receptor. VEGF has three structurally similar type of receptor tyrosine kinases, designated VEGFR1 (also known as FLT1), VEGFR2 (also known as KDR) and VEGFR3 (also known as FLT4) (Figure 3.1), which differ considerably in signaling process. VEGF receptors have seven immunoglobulin-like domains in the extra cellular part, a single transmembrane domain and a tyrosine kinase domain in the intra cellular part mostly found in endothelial cells.

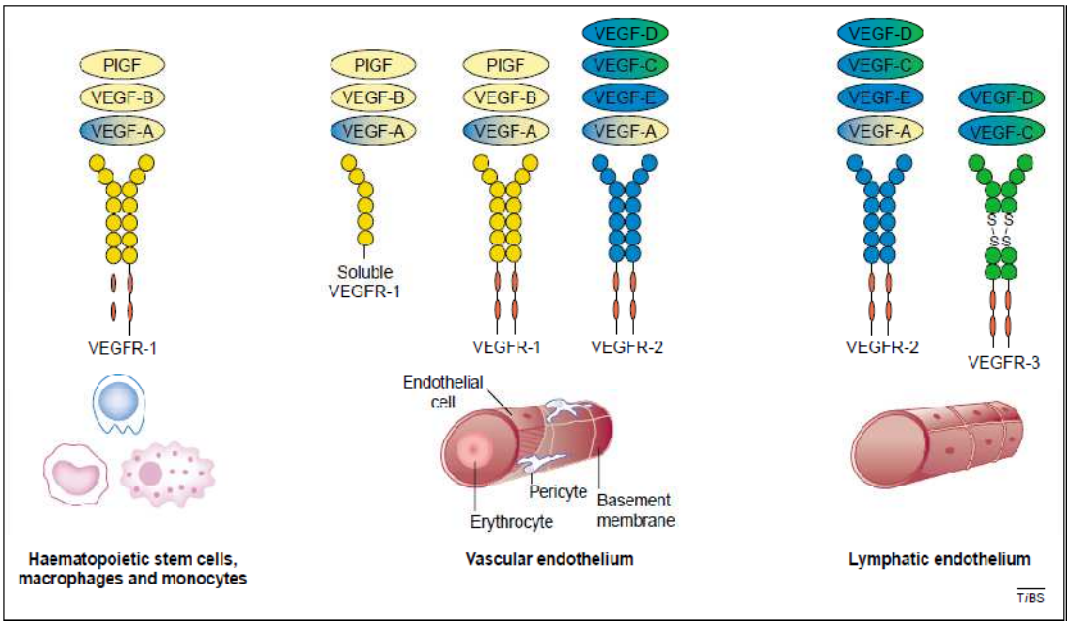


Figure 3.1 Schematic illustration of VEGF and VEGFR expression pattern and ligand specificity (Cross et al., 2003).

Circulating VEGF binds to VEGF receptors (VEGFR). This ligand-receptor engagement triggers the signaling pathways for the activation of endothelial cells. Activation and subsequent proliferation of endothelial cells are prerequisite for angiogenesis since newly proliferated endothelial cells participate in the formation of proximal new blood vessels. The molecules secreted from activated endothelium include vascular cell adhesion molecule-1 (VCAM-1) and matrix metalloproteinases (MMPs). VEGF also acts as a proinflammatory cytokine by increasing endothelial permeability and inducing adhesion molecules that bind leukocytes to endothelial cells (Detmar et al., 1998; Melder et al., 1996). The distinct signal transduction mechanisms by which VEGF induces survival, proliferation, migration, and nitric oxide (NO) production in endothelial cells have been identified (Kim et al., 2001). Previously, Hossain et al. (2012) reported that arsenic exposure increases plasma Big endothelin-1 (Big ET-1), a precursors of endothelin-1. Plasma Big ET-1 is a specific marker of endothelial activation/dysfunction. Further, positive association of arsenic exposure with the plasma VCAM-1 levels in arsenic-endemic population in Bangladesh was observed (Chapter 2). Although these previous studies provide evidence that arsenic exposure is associated endothelial activation/dysfunction, biochemical events in arsenic-mediated activation of endothelium in human remains to be clarified. Binding of circulating VEGF to its receptors causes the activation of endothelial cells which ultimately lead to the secretion of several molecules required for the proliferation of these cells. Experimental studies have examined the effect of arsenic exposure on VEGF expression using cultured cell lines. Duyndam et al. (2001) have shown that arsenic increases VEGF expression in both human umbilical vein endothelial cells and cervical cancer cells. Soucy et al. (2004) have reported that arsenic exposure causes VEGF expression in smooth muscle cells dose-dependently. More recently, Liu et al. (2011) has reported that arsenic exposure induces angiogenesis through VEGF expression in both human immortalized lung epithelial cells and adenocarcinoma cells. In contrast, the other groups have reported that arsenic exposure causes the inhibition of VEGF expression in various cell lines (Roboz et al., 2000; Xiao et al., 2006; Yu et al., 2006). Therefore, contradictory results in cell line experiments have necessitated epidemiological studies to clarify the association of arsenic exposure and serum VEGF levels.

Several epidemiological studies have established the relationship of cancer and vascular diseases with VEGF (Di Raimondo et al., 2001; Kimura et al., 2007; Poon et al., 2001). Arsenic exposure has been shown to be associated with a variety of cancers and several forms of vascular diseases such as atherosclerosis, ischemic heart disease and peripheral vascular disease (Chen and Ahsan 2004; Chen et al., 2011; Chiou et al., 1995; Hossain et al., 2012; Smith, 2009; Tseng, et. al., 1996). However, mechanistic insights into the vulnerability of arsenic-exposed individuals to cancers and vascular diseases are largely unknown. It is expected that arsenic exposure causes the expression of VEGF or other molecules making proangiogenic micro environment favourable for the development of tumor and vascular diseases in humans. In an attempt to investigate the proangiogenic effect of long-term arsenic exposure, this research for the first time explored the association between arsenic exposure and serum VEGF levels in human exposed to arsenic chronically in Bangladesh.

3.2 Materials and Methods

3.2.1 Ethical permission

Ethical permission for the human study was granted by the Institute of Biological Sciences, University of Rajshahi, Bangladesh. The subjects who participated in this study gave their written consent. All sorts of confidentialities and rights of the study subjects were strictly maintained.

3.2.2 Selection of study areas and subjects

Study areas were selected from the north-west region of Bangladesh. The study areas included Marua in Jessore, Dutpatila, Jajri, Vultie and Kestopur in Chuadanga, and Bheramara in Kushtia districts of Bangladesh. Study areas and study subjects were chosen as described previously in the materials and methods section of chapter 2. Of the 267 individuals recruited, seven individuals were excluded according to the exclusion criteria [i.e., study candidates (n=3) who had resided in the arsenic-endemic areas for less than 5 years, pregnant and lactating mothers (n=2), and had hepatological diseases (n=2)]. Thus, a total of 260 individuals were finally participated.

3.2.3 Water collection and arsenic analysis

Water samples were collected in acid-washed containers from the tube wells which the study subjects are using as a primary source of drinking water as described in materials and method section of chapter 2. Total arsenic concentration in water samples was determined by ICP-MS as described previously (Chapter 2). River water was used as a CRM to confirm the accuracy of water arsenic concentration determined by ICP-MS.

3.2.4 Collection of hair and nail samples, and analysis of arsenic

Collection of hair and nail samples from each study subject and analysis of total arsenic concentrations in both hair and nails samples were determined by ICP-MS as described previously in the materials and method section (chapter 2).

3 .2.5 Collection of blood serum

All study subjects were requested to fast overnight (10–12 h) to collect fasting blood samples. Blood samples (5-7 ml) were left at room temperature for 30 minutes for clotting and were subsequently centrifuged at 1,200 × g for 20 minutes. The serum supernatant was then taken and stored at - 80°C.

(A) **(B)** **(C)**

(D) **(E)** **(F)**

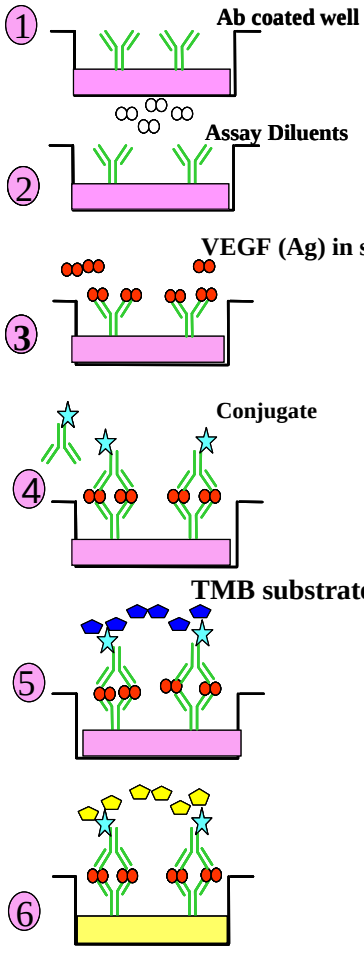
Figure 3.2 Some representative photographs of field activities. **(A)** A visit to the study area, **(B)** and **(C)** Personal interview, **(D)** BMI measurement, **(E)** Physical examination of a study subject by a physician, **(F)** Blood sample collection.

3.2.6 Measurements of serum VEGF

Serum levels of $VEGF_{165}$ were measured using commercially available sandwich enzyme-linked immunoassay kits (R&D Systems, Inc. Minneapolis, USA) according to the manufacture's protocols. On completion of the assay, the observed color change was measured using a microplate reader (Mikura Ltd. UK) and VEGF level was calculated by extrapolation from a standard curve. A separate standard curve was constructed for each immunoassay. All standards and samples were analyzed in duplicate and the mean values were taken. The intra and inter assay coefficients of variations (CVs) were maximum 10%. According to the manufacturer's protocol the sensitivity and the assay range of serum VEGF were 9.0 pg/mL and 31.2-2000 pg/mL, respectively.

Basic principle for VEGF assay: VEGF assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for VEGF was pre-coated onto a microplate. Standards and samples were pipette into the wells and any VEGF present was bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for VEGF was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells and color developed in proportion to the amount of VEGF bound in the initial step. The color development was stopped by adding acid and the intensity of the color was measured at 450 nm.

Stepwise assay:



1 Anti-VEGF antibody (Ab) coated well

2 Assay diluents was added to anti-VEGF coated well

3 Serum was added to plate and antigen (VEGF) present was bound to antibody (Ab)

4 Addition of HRP labelled polyclonal anti-VEGF Ab (conjugate) which also bound to the antigen (Ag) present in the plate

5 TMB substrate was added to the plate

6 Substrate was converted by HRP to a detectable form (from blue to yellow

7 Detection by microplate reader

7 Intensity of the color was measured by microplate reader

Figure 3.3 Stepwise assay of VEGF by ELISA kit

3.2.7 Statistical analysis

Statistical analysis for this study was performed using software of Statistical Packages for Social Sciences (SPSS version 17.0, SPSS Inc., Chicago, IL) after log transformation of the values of water, hair and nail arsenic, and levels of serum VEGF due to the skewed distribution of the raw data. Normality of the distribution of variables was verified by a Q-Q plot. The study subjects were divided into three groups (low, medium and high) with approximately equal population size based on arsenic levels in water, hair and nails. In addition, the study subjects were divided into three groups (≤ 10 μg/L, 10.1-50 μg/L and > 50 μg/L) based on the regulatory upper limit of water arsenic concentration set by WHO, (10 μg/L) and Bangladesh Government (50 μg/L). The mean age, arsenic concentration, monthly income and BMI of the study subjects in low, medium, and high groups of water arsenic concentrations were compared by *F*-test (One-way ANOVA), whereas the smoking status, occupation, and education were compared by Chi-square test. Spearman correlation coefficient tests were used to evaluate the correlations of arsenic exposure metrics with serum VEGF levels. Dose-response relationships of arsenic exposure metrics with serum VEGF levels were analyzed by One-way ANOVA followed by Bonferroni's multiple comparison test. Finally, Univariate linear regression analyses were used to examine the associations of arsenic exposure metrics with serum VEGF levels after adjusting for age, sex, BMI, and smoking. A value of $p<0.05$ was considered statistically significant.

3.3 Results

3.3.1 Characteristics of the study subjects

Table 3.1 shows the characteristics of the study subjects in the low, medium and high exposure groups depending on the concentrations of arsenic in drinking water. There were total 127 female and 133 male subjects with a mean age of 38.56 ± 12.35 years. The concentrations of water, hair and nail arsenic in the low, medium and high groups were significantly different. Most of the female study subjects were house wives (93.70%) and remaining 6.30% were farm workers and students, and most of the male subjects were farmers (81.20%) and remaining 18.80% were students, businessman, and others (small vendors, rickshaw pullers, and tailors). Socioeconomic characteristics (occupation, monthly income and education) of the

study subjects in each group were almost similar. The percentage of the study individuals who completed primary education were 26.74, 39.53 and 30.68 in the low, medium and high exposure groups, respectively. The numbers of non-smoker in each group were higher than those of smokers. We did not find any female smoker as generally Bangladeshi women do not smoke cigarette. No study subject had admitted to drink alcohol. This is because of the social and religious restriction on drinking of alcohol. The averages of BMI (mean ± SD) of the three exposure groups were almost similar.

Table 3.1 Characteristics of the study subjects based on water arsenic concentrations

Characteristics	All (0.29 - 546 µg/L)	Low (0.29 – 57.01 µg/L)	Medium (57.26 – 205 µg/L)	High (214 - 546 µg/L)	*p*-value 0
No. of subjects	260	86	86	88	
Sex [no. (female/male)]	127/133	35/51	47/39	45/43	
Age [years (mean ± SD)]	38.56 ± 12.35	38.90 ± 13.27	39.97 ± 12.44	36.86 ± 11.22	0.243*
Water As concentration (µg/L) Geometric mean(SD)	70.22 (6.23)	8.22 (4.87)	121 (1.43)	335.92 (1.31)	<0.001*
Hair As concentration (µg/g) Geometric mean (SD)	2.91 (2.66)	1.6 (2.54)	3.29 (2.22)	4.64 (2.44)	<0.001*
Nail As concentration (µg/g) Geometric mean (SD)	7.08 (2.24)	4.8 (2.23)	7.65 (2.08)	9.58 (2.06)	<0.001*
Occupation [n, (%)]					
Female					
Housewife	119 (93.70)	35 (100)	45 (95.74)	39 (86.67)	0.719†
Farm worker	6 (4.72)	0	2 (4.26)	4 (8.89)	
Student	2 (1.57)	0	0	2 (4.44)	
Male					
Farmer	108 (81.20)	40 (78.43)	32 (82.05)	36 (83.72)	
Student	4 (3.01)	3 (5.88)	1 (2.56)	0	
Business	4 (3.01)	2 (3.92)	0	2 (4.65)	
Others	17 (12.78)	6 (11.76)	6 (15.38)	5 (11.63)	
Education [n, (%)]					
No formal education	151 (58.08)	49 (56.98)	46 (53.49)	56 (63.64)	0.124†
Primary	84 (32.31)	23 (26.74)	34 (39.53)	27 (30.68)	
Secondary	24 (9.23)	13 (15.12)	6 (6.98)	5 (5.68)	
Graduate	1 (0.38)	1 (1.16)	0	0	
Monthly income (U.S.$)	26.09 ± 14.22	25.12 ± 13.18	26.36 ± 17	26.71 ± 12.22	0. 742*
Smoking habit [n, (%)]					
Yes	57 (21.92)	21 (24.42)	20 (23.26)	16 (18.18)	0.571†
No	203 (78.08)	65 (75.58)	66 (76.74)	72 (81.82)	
Alcohol intake	-	-	-	-	-
BMI[b] (mean ± SD)	20.51 ± 3.23	20.69 ± 3.18	20.63 ± 3.00	20.22 ± 3.50	0.571*

Data were presented as mean ± SD, and geometric mean (SD) for log transformed value. Abbreviation: As= Arsenic, BMI[b] = Body Mass Index was calculated as body weight (kg) divided by body height squared (m^2); *p-, and †p–values were from the one way ANOVA (F-test) and Chi-square test, respectively.

3.3.2 Correlation of water arsenic with hair and nail arsenic concentrations

First, correlations of external arsenic exposure metric (drinking water) with internal exposure metrics (hair and nails) were checked. Water arsenic concentrations showed a strong positive correlation (r_s = 0.811, p< 0.001) with hair arsenic concentrations (Figure 3.4). A similar positive relationship (r_s = 0.772, p< 0.001) was also observed between water and nail arsenic concentrations (Figure 3.4). All these strong positive associations suggested that drinking water arsenic was the major source of accumulated arsenic in hair and nails of the study subjects.

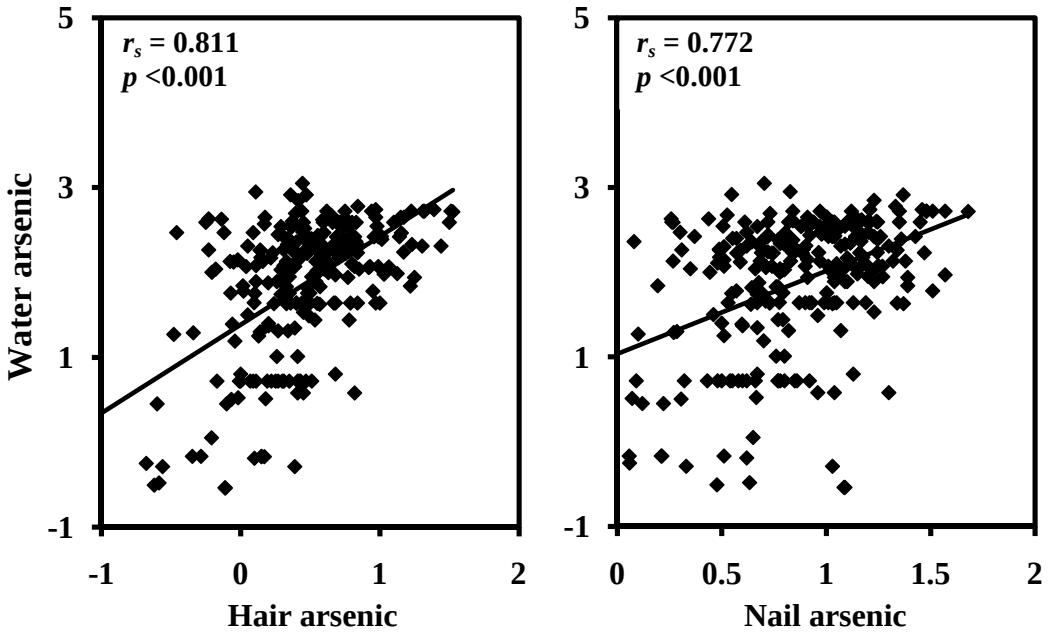


Figure 3.4 Correlations of water arsenic with hair and nail arsenic concentrations. Log-transformed values of arsenic concentrations were used. r_s and *p*-values were from Spearman correlations coefficient test.

3.3.3 Correlation between arsenic exposure and serum VEGF levels

Figure 3.5 shows the correlation between arsenic exposure metrics (water, hair and nail arsenic concentrations) and serum VEGF levels. A significant increase in serum VEGF levels was observed with the increasing concentrations of total arsenic in drinking water (r_s = 0.363, p<0.001). Almost similar relationships were also observed between serum VEGF levels and hair arsenic concentrations (r_s = 0.205, p<0.01), and between serum VEGF levels and nail arsenic concentrations (r_s = 0.190, p<0.01).

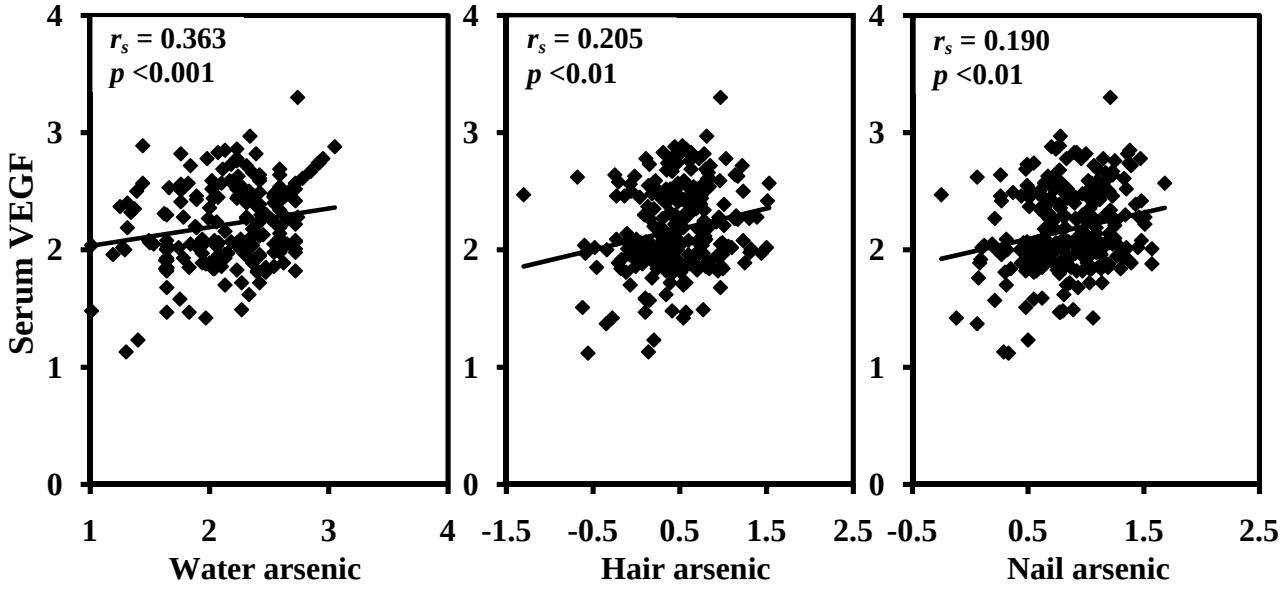


Figure 3.5 Correlation between arsenic exposure and serum VEGF levels. Log-transformed values of arsenic concentrations and serum VEGF were used. r_s and p-values were from Spearman correlations coefficient test.

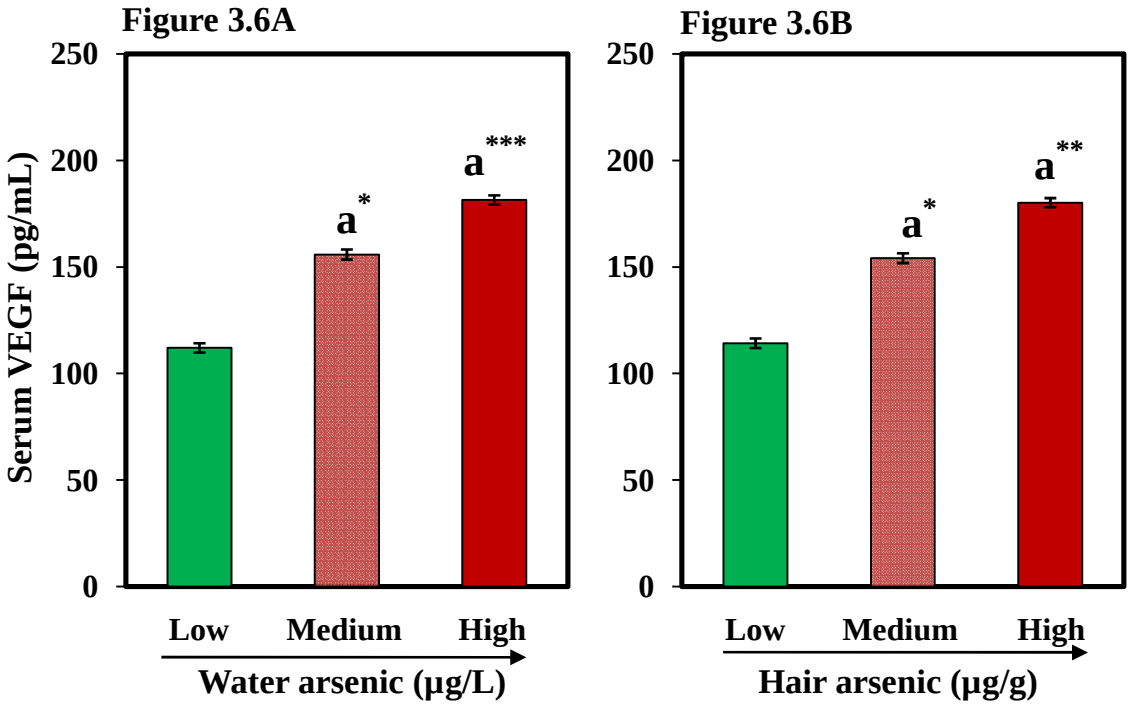
3.3.4 Dose-response relationship of arsenic exposure with serum VEGF levels

Figure 3.6 shows the dose-response relationship of arsenic exposure with serum VEGF levels. At first, serum VEGF levels were measured in tertile (low, medium and high) groups based on the three concentrations of water arsenic (external exposure metric). Serum VEGF levels were gradually increased in the higher exposure gradients, however, the differences were statistically significant in medium versus low (p< 0.05), and high versus low (p< 0.001) exposure groups (Figure 3.6A). Next, dose-response relationships of hair and nail arsenic concentrations (internal exposure metrics) with serum VEGF levels were checked. Serum VEGF levels were found to

be higher in the higher exposure gradients but the differences were significant in the medium versus low ($p < 0.05$), and high versus low ($p < 0.01$) exposure groups of hair and nail arsenic concentrations (Figure 3.6B and 3.6C).

3.3.5 Comparison of serum VEGF levels in the three groups based on the regulatory upper limit of arsenic concentrations in drinking water

Next, dose-response relationship was evaluated among the three groups (≤10 µg/L, 10.1-50 µg/L and >50 µg/L) based on the regulatory upper limit of water arsenic concentrations set by WHO (10 µg/L) and Bangladesh Government (50 µg/L). Serum VEGF levels were significantly higher in >50 µg/L group compared to ≤10 µg/L groups. Serum VGEF levels were also found to be higher in 10.1-50 µg/L group than ≤10 µg/L group but the difference was not statistically significant (Figure 3.7).



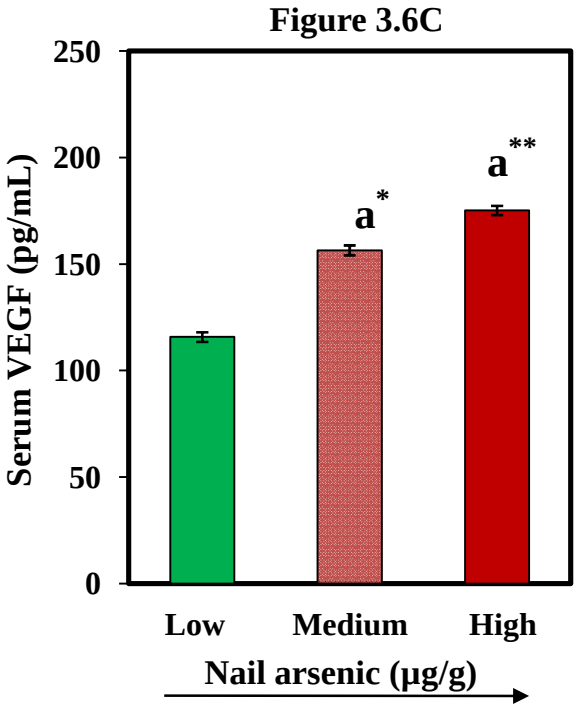


Figure 3.6 Dose-response relationships of serum VEGF levels with arsenic exposure metrics. Data were presented as geometric mean (SD). *p*-values were from one-way ANOVA followed by Bonferroni multiple comparison test between each group of exposure level. Arsenic levels in water; low (0.29 - 57.01 µg/L; n = 86), medium (57.26 - 205 µg/L; n = 86), and high (214 - 546 µg/L; n = 88). Arsenic levels in hair; low (0.05 - 2.14 µg/g; n = 86), medium (2.16 - 4.20 µg/g; n = 86), and high (4.26 - 33.51 µg/g; n = 88). Arsenic levels in nail; low (0.56 - 5.02 µg/g; n = 86), medium (5.06 - 10.78 µg/g; n = 86), and high (10.85 - 47.83 µg/g; n = 88).
[a]Statistically significant from low group**.** ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$.

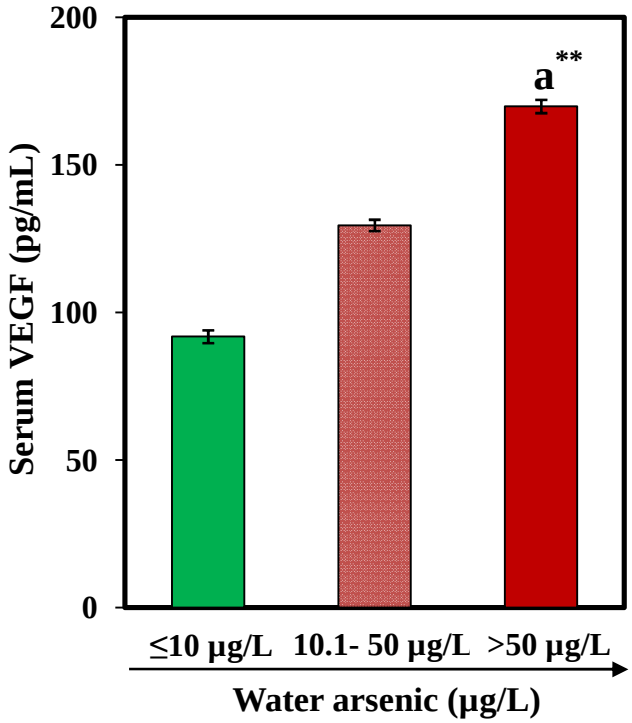


Figure 3.7 Serum VEGF levels in the three groups based on the regulatory upper limits of arsenic concentrations in drinking water. Data were presented as geometric mean (SD). *p*-values were from one-way ANOVA followed by Bonferroni multiple comparison test between each group of exposure level.
[a]Statistically significant from ≤ 10 µg/L group. **, $p < 0.01$

3.3.6. Effect of covariates on serum VEGF levels

Univariate linear regression analyses were performed to evaluate the effects of age, sex, BMI, and smoking as covariates on the arsenic-induced elevation of serum VEGF. Table 3.2 shows the results of univariate linear regression analyses on the association of serum VEGF levels (dependent variable) with arsenic exposure metrics (independent variable) after adjusting with these covariates. Water, hair and nail arsenic concentrations showed significant contribution to serum VEGF levels as evidenced by β-coefficient values.

Table 3.2 Associations of arsenic exposure with serum VEGF levels through regression analysis

Independent Variable	Dependent Variable Serum VEGF		
		Before adjustment	**After adjustment**
Water arsenic	β-Coefficient (95% CI)	0.160 (0.109 - 0.211)	0.163 (0.111 - 0.215)
	p-value	<0.001	<0.001
Hair arsenic	β-Coefficient (95% CI)	0.177 (0.076 - 0.278)	0.181 (0.077 - 0.284)
	p-value	<0.01	<0.01
Nail arsenic	β-Coefficient (95% CI)	0.225 (0.102 - 0.348)	0.232 (0.107 - 0.357)
	p-value	<0.001	<0.001

Abbreviation: CI= Confidence interval; Log-transformed values of arsenic and serum VEGF were used. Data were adjusted for age, sex, BMI, and smoking.

3.4. Discussion

Several epidemiological studies have shown that arsenic exposure is associated with the development of variety of solid tumors as well as cancers (Chen and Ahsan, 2004; Chiou et al., 1995; Smith, 2009)**.** International Agency for Research on Cancer (IARC) has classified arsenic as a Group-1 human carcinogen. To the best of our knowledge this is the first human study to show the association between arsenic exposure and serum VEGF levels. VEGF is a key molecule of angiogenesis which is deeply implicated in cancer, various ischemic and inflammatory diseases. Arsenic is a well established human carcinogen that causes a variety of cancers. Cancer is one of the major causes of arsenic-related morbidity and mortality (Lokuge et al., 2004). This study demonstrated that arsenic exposure was significantly correlated with the increased serum VEGF levels in arsenic-endemic population in Bangladesh (Figure3.5). Furthermore, serum VEGF levels showed dose-response relationships with arsenic exposure metrics including water, hair, and nail arsenic concentrations (Figure 3.6 and 3.7).

No human study has ever shown the relationship between arsenic exposure and serum VEGF levels. However, several studies have examined the relationship of arsenic exposure with VEGF expression using cultured cell lines. Increased levels of serum VEGF with the arsenic exposure observed in this study were in good agreement with the results of cell line experiments reported by Duyndam et al. (2001),

Liu et al. (2011) and Soucy et al. (2004). However, opposite results have been reported by other groups in various cell lines (Roboz et al., 2000; Xiao et al., 2006; Yu et al., 2006). The reasons for such discrepancies might be due to the differences in cell types and concentrations of arsenic used. Translation of laboratory arsenic toxicology studies to human health is important but it is complicated by exact dose conversion between in vitro, animal, and human exposure and species-specific metabolic and genetic differences. In fact no suitable animal model for arsenic toxicity has been developed yet that can well correspond to the toxic effects of arsenic exposures observed in humans. Therefore, this human study showing association and exposure-response relationship between arsenic exposures and serum VEGF levels is noteworthy.

In normal physiological conditions, there is a balance between pro- and anti-angiogenic molecules. The angiogenic switch is 'off' when the effect of pro-angiogenic molecules is balanced by that of anti-angiogenic molecule, is 'on' when the net balance is tripped in favour of angiogenesis (Bouck et al., 1996; Neiberg et al., 2005). Therefore, the positive correlations and dose-response relationships of serum VEGF levels with arsenic exposure metrics provide evidence in support of shifting physiological balance towards pro-angiogenic environment by arsenic exposure.

In this study, the mechanisms how arsenic increased the levels of VEGF in serum remain to be clarified. Several in vitro studies, however, have proposed the mechanisms of arsenic-induced VEGF expression (Bao et al., 2010; Duyndam et al., 2001). Arsenic induces VEGF expression through hypoxia inducible factor-1α (HIF-1α) in human prostate cancer and ovarian cancer cells (Duyndam et al., 2001; Gao et al., 2004). HIF-1α-mediated VEGF expression depends mainly on reactive oxygen species (ROS), which enhances transcription of the gene encoding VEGF (Carpenter et al., 2005; Fisher et al., 1999; Maulik and Das 2002). Activating transcription factor 4 (ATF4) has been found to be another mediator for VEGF expression. Arsenic also causes ATF4 expression through redox-linked mechanism (Roybal et al., 2005). On the other hand, Meng et al. (2010) showed that arsenic-induced heme oxygenase-1 is able to stimulate VEGF expression in human micro vascular endothelial cells through ROS-independent pathway.

VEGF has been recognized as a reliable surrogate marker of angiogenic activity for tumor progression (Poon et al., 2001). In addition to the tumor angiogenesis,

VEGF has been implicated in the pathophysiology of many diseases such as ischemic diseases, atherosclerosis and diabetes because of the ability of VEGF for increasing vascular permeability (Weis and Cheresh 2005). Therefore, strong correlation and dose-dependent association of arsenic exposure with serum VEGF levels observed in this study shed light, at least in part, on the mechanistic insight into the development of cancer and vascular diseases in humans exposed to arsenic.

There are several unique features in the present study. First, all the associations were found across the three kinds of exposure metrics (water, hair and nail arsenic concentrations). As we reported previously (Ali et al., 2010; Hossain et al., 2012), in this study we also found that these three kinds of exposure metrics were strongly correlated with each other (Figure 3.4). Therefore, the assessment of arsenic exposure by three kinds of exposure metrics and their correlations with VEGF may exclude the possibilities of miss classification. Second, a good number study population with a wide variation of arsenic exposure levels that showed the precise nature of dose-response relationship between arsenic exposure and serum VEGF levels. Further dose-response relationship among the three groups based on the permissive limit of water arsenic set by WHO and Bangladesh Government showed that serum VEGF levels in 10.1-50 µg/L group were higher than in ≤10 µg/L group. The differences were not statistically significant. However, the result might be of note from policy perspectives as the permissive limit set by the Bangladesh Government is 5 times higher than that of the WHO. There are several limitations in our study that warrant further discussion. First, although we showed the association between the arsenic exposure and serum VEGF levels before and after adjusting for age, sex, BMI, and smoking habits (Table 3.2), there may be some other factors such as co-exposure to other metals, insecticides or pesticides or individual variations that could influence the VEGF levels. If any accompanying metals or other contaminants could influence the observed associations, then they would also be expected to follow the same concentration gradients as arsenic in the drinking water, hair and nails. This is unlikely, but more extensive study of the other metals and their association with serum VEGF levels are required in future. Second, this study was designed to be cross sectional, but not prospective. A cohort based study is needed in future to verify the cause-effect relationship between arsenic exposure and serum VEGF levels. Third, most of our study individuals were at the lower end of normal range of BMI (Table 3.1). Thus, the results of the current study may not be generalizable to other study populations and the study needs to be replicated in other population.

3.5 Conclusion

The present study showed that serum VEGF levels significantly correlated with increasing concentrations of arsenic in drinking water, hair and nails of the study individuals exposed to arsenic chronically in Bangladesh. Dose-response relationships between arsenic exposure and serum VEGF levels have been observed. Further, dose-response relationship were also observed among the three groups (≤10 µg/L, 10.1-50 µg/L and >50 µg/L) based on the regulatory upper limit of water arsenic concentrations set by WHO (10 µg/L) and Bangladesh Government (50 µg/L). The associations of arsenic exposure with serum VEGF levels observed in this study remain significant even after adjusting for age, sex, BMI, and smoking as covariates. Thus, the results of this study provide evidence in support of the proangiogenic effect of arsenic exposure on humans that may induce favourable physiological environment for the initiation and progression of tumors and vascular diseases.

3.6 References

Ali, N., Hoque M. A., Haque, A., Salam, K. A., Karim, M. R., Rahman, A., Islam, K., Saud, Z. A., Khalek M. A., Akhand, A. A., Hossain, M., Mandal, A., Karim, M. R., Miyataka, H., Himeno, S., and Hossain, K. (2010). Association between arsenic exposure and plasma cholinesterase activity: a population based study in Bangladesh. *Environ. Health* **9**, 36.

Banks, R. E., Forbes, M. A., Kinsey, S. E., Stanley, A., Ingham, E., Walters, C., and Selby, P. J. (1998). Release of the angiogenic cytokine vascular endothelial growth factor (VEGF) from platelets: significance for VEGF measurements and cancer biology. *Br. J. Cancer* **77**, 956-964.

Bao, L., and Shi, H. (2010). Arsenite induces endothelial cell permeability increase through a reactive oxygen species-vascular endothelial growth factor pathway. *Chem. Res. Toxicol.* **23**, 1726-1734.

Bouck, N., Stellmach. V., and Hsu, S. C. (1996). How tumors become angiogenic. *Adv. Cancer Res*. **69,** 135-174.

Caldwell, B. K., Caldwell, J. C., Mitra, S. N., and Smith, W. (2003). Tube wells and arsenic in Bangladesh: challenging a public health success story. *Int. J. Popul. Geogr*. **9**, 23-38.

Carmeliet, P., and Collen, D. (2000). Molecular basis of angiogenesis. Role of VEGF and VE-cadherin. *Ann. N. Y. Acad. Sci*. **902**, 249-262.

Carpenter, T. C., Schomberg, S., and Stenmark, K. R. (2005). Endothelin-mediated increases in lung VEGF content promote vascular leak in young rats exposed to viral infection and hypoxia. *Am. J. Physiol. Lung Cell Mol. Physiol*. **289**, L1075-L1082.

Chen, C. J., Wang, S. L., Chiou, J. M., Tseng, C. H., Chiou, H. Y., Hsueh, Y. M., Chen, S. Y., Wu, M. M., and Lai, M. S. (2007). Arsenic and diabetes and hypertension in human populations: a review. *Toxicol. Appl. Pharmacol*. **222**, 298-304.

Chen, Y., and Ahsan, H. (2004). Cancer burden from arsenic in drinking water in Bangladesh. *Am. J. Public Health* **94**, 741-744.

Chen, Y., Graziano, J. H., Parvez, F., Liu, M., Slavkovich, V., Kalra, T., Argos, M., Islam, T., Ahmed, A., Rakibuz-Zaman, M., Hasan, R., Sarwar, G., Levy, D., van Geen, A., and Ahsan, H. (2011). Arsenic exposure from drinking water and mortality from cardiovascular disease in Bangladesh: prospective cohort study. *BMJ*. **342**, d2431.

Chiou, H. Y., Hsueh, Y. M., Liaw, K. F., Horng, S. F., Chiang, M. H., Pu, Y. S., Lin, J. S., Huang, C. H., and Chen, C. J. (1995). Incidence of internal cancers and ingested inorganic arsenic: a seven-year follow-up study in Taiwan. *Cancer Res*. **55**, 1296-1300.

Chowdhury, A. M. R. (2004). Arsenic crisis in Bangladesh. *Sci. Am*. **291**, 86-91.

Chowdhury, U. K., Biswas, B. K., Chowdhury, T. R., Samanta, G., Mandal, B. K., Basu, G. C., Chanda, C. R., Lodh, D., Saha, K. C., Mukherjee, S. K., Roy, S., Kabir, S., Quamruzzaman, Q., and Chakraborti, D. (2000). Groundwater arsenic contamination in Bangladesh and West Bengal, India. *Environ. Health Perspect*. **108**, 393-397.

Cross, M. J., Dixelius, J., Matsumoto, T., and Claesson-Welsh, L. (2003). VEGF-receptor signal transduction. *Trends. Biochem. Sci*. **28**, 488-494.

Detmar, M., Brown, L. F., Schon, M. P., Elicker, B. M., Velasco, P., Richard, L., Fukumura, D., Monsky, W., Claffey, K. P, and Jain, R. K. (1998). Increased microvascular density and enhanced leukocyte rolling and adhesion in the skin of VEGF transgenic mice. *J. Invest. Dermatol*. **111**, 1-6.

Di Raimondo, F., Azzaro, M. P., Palumbo, G. A., Bagnato, S., Stagno, F., Giustolisi, G. M., Cacciola, E., Sortino, G., Guglielmo, P., and Giustolisi, R. (2001). Elevated vascular endothelial growth factor (VEGF) serum levels in idiopathic myelofibrosis. *Leukemia* **15**, 976-780.

Duyndam, M. C., Hulscher, T. M., Fontijn, D., Pinedo, H. M., and Boven, E. (2001). Induction of vascular endothelial growth factor expression and hypoxia-inducible factor 1alpha protein by the oxidative stressor arsenite. *J. Biol. Chem*. **276**, 48066-48076.

Ferrara, N. (2002). VEGF and the quest for tumour angiogenesis factors. *Nat. Rev. Cancer* **2**, 795-803.

Fischer, S., Clauss, M., Wiesnet, M., Renz, D., Schaper, W., and Karliczek, G. F. (1999). Hypoxia induces permeability in brain micro vessel endothelial cells via VEGF and NO. *Am. J. Physiol*. **276**, C812-C820.

Gao, N., Shen, L., Zhang, Z., Leonard, S. S., He, H., Zhang, X. G., Shi, X., and Jiang, B. H. (2004). Arsenic induces HIF-1 alpha and VEGF through PI3K, Akt and reactive oxygen species in DU145 human prostate carcinoma cells. *Mol. Cell Biochem*. **255**, 33-45.

Gunsilius, E., Petzer, A., Stockhammer, G., Nussbaumer, W., Schumacher, P., Clausen, J., and Gastl, G. (2000). Thrombocytes are the major source for soluble vascular endothelial growth factor in peripheral blood. *Oncology* **58**, 169-174.

Hossain, E., Islam, K., Yeasmin, F., Karim, M. R., Rahman, M., Agarwal, S., Hossain, S., Aziz, A., Mamun, A. A., Sheikh, A., Haque, A., Hossain, M. T., Hossain, M., Haris, P. I., Ikemura, N., Inoue, K., Miyataka, H., Himeno, S., and Hossain, K. (2012). Elevated levels of plasma Big endothelin-1 and its relation to hypertension and skin lesions in individuals exposed to arsenic. *Toxicol. Appl. Pharmacol.* **259**, 187-194.

Houck, K. A., Ferrara, N., Winer, J., Cachianes, G., Li, B., and Leung, D. W. (1991). The vascular endothelial growth factor family: identification of a fourth molecular species and characterization of alternative splicing of RNA. *Mol. Endocrinol*. **5**, 1806-1814.

Islam, K., Haque, A., Karim, M. R., Fajol, A., Hossain, E., Salam, K. A., Ali, N., Saud, Z. A., Rahman, M., Rahman, M., Karim, R., Sultana, P., Hossain, M., Akhand, A. A., Mandal, A., Miyataka, H., Himeno, S., and Hossain, K. (2011). Dose-response relationship between arsenic exposure and the serum enzymes for liver function tests in the individuals exposed to arsenic: a cross sectional study in Bangladesh. *Environ. Health* **10**, 64.

Kim, I., Moon, S. O., Kim, S. H., Kim, H. J., Koh, Y. S., and Koh, G. Y. (2001). Vascular endothelial growth factor expression of intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and E-selectin through nuclear factor-kb activation in endothelial cells. *J. Biol. Chem.* **276**, 7614-7620.

Kimura, K., Hashiguchi, T., Deguchi, T., Horinouchi, S., Uto, T., Oku, H., Setoyama, S., Maruyama, I., Osame, M., and Arimura, K. (2007). Serum VEGF-as a prognostic factor of atherosclerosis. *Atherosclerosis* **194**, 182-188.

Lai, M. S., Hsueh, Y. M., Chen, C. J., Shyu, M. P., Chen, S. Y., Kuo, T. L., Wu, M. M., and Tai, T. Y. (1994). Ingested inorganic arsenic and prevalence of diabetes mellitus. *Am. J. Epidemiol*. **139**, 484-492.

Leung, D. W., Cachianes, G., Kuang, W. J., Goeddel, D. V., and Ferrara, N. (1989). Vascular endothelial growth factor is a secreted angiogenic mitogen. *Science* **246**, 1306-1309.

Liu, L. Z., Jiang, Y., Carpenter, R. L., Jing, Y., Peiper, S. C., and Jiang, B. H. (2011). Role and mechanism of arsenic in regulating angiogenesis. *PLoS* One **6**, e20858.

Lokuge, K. M., Smith, W., Caldwell, B., Dear, K., and Milton, A. H. (2004). The effect of arsenic mitigation interventions on disease burden in Bangladesh. *Environ. Health Perspect*. **112**, 1172-1177.

Maulik, N., and Das, D. K. (2002). Redox signaling in vascular angiogenesis. *Free Radical Biol. Med.* **33**, 1047-1060.

Mazumder, D. N. G., Haque, R., Ghosh, N., De, B. K., Santra, A., Chakraborty, D., and Smith, A. H. (1998). Arsenic levels in drinking water and the prevalence of skin lesions in West Bengal, India. *Int. J. Epidemiol*. **27**, 871-877.

Melder, R. J., Koenig, G. C., Witwer, B. P., Safabakhsh, N., Munn, L. L., and Jain, R. K. (1996). During angiogenesis, vascular endothelial growth factor and basic fibroblast growth factor regulate natural killer cell adhesion to tumor endothelium. *Nat. Med.* **2**, 992-997.

Meliker, J. R., Wahl, R. L., Cameron, L. L., and Nriagu, J. O. (2007). Arsenic in drinking water and cerebrovascular disease, diabetes mellitus, and kidney disease in Michigan: a standardized mortality ratio analysis. *Environ. Health*. **4**, 6.

Meng, D., Wang, X., Chang, Q., Hitron, A., Zhang, Z., Xu, M., Chen, G., Luo, J., Jiang, B., Fang, J., and Shi, X. (2010). Arsenic promotes angiogenesis in vitro via a heme oxygenase-1-dependent mechanism. *Toxicol. Appl. Pharmacol*. **244**, 291-299.

Mohle, R., Green, D., Moore, M.A., Nachman, R. L., and Rafii, S. (1997). Constitutive production and thrombin-induced release of vascular endothelial growth factor by human megakaryocytes and platelets. *Proc. Natl. Acad. Sci.* **94**, 663-668.

Neufeld, G., Cohen, T., Gengrinovitch, S. and Poltorak, Z. (1999). Vascular endothelial growth factor (VEGF) and its receptors. *FASEB J*. **13**, 9-22.

Nyberg, P., Xie, L., and Kalluri, R. (2005). Endogenous inhibitors of angiogenesis. *Cancer Res*. **65,** 3967-3979.

Poon, R. T., Fan, S. T., and Wong, J. (2001). Clinical implications of circulating angiogenic factors in cancer patients. *J. Clin. Oncol*. **19**, 1207-1225.

Roboz, G. J., Dias, S., Lam, G., Lane, W. J., Soignet, S. L., and Rafii, S. (2000). Arsenic trioxide induces dose- and time-dependent apoptosis of endothelium and may exert an antileukemic effect via inhibition of angiogenesis. *Blood* **96**, 1525-1530.

Roybal, C. N., Hunsaker, L. A., Barbash, O., Vander Jagt, D. L., and Abcouwer, S. F. (2005). The oxidative stressor arsenite activates vascular endothelial growth factor mRNA transcription by an ATF4-dependent mechanism. *J. Biol. Chem*. **280**, 20331-20339.

Shinkaruk, S., Bayle, M., Laïn, G., and Déléris, G. (2003). Vascular endothelial cell growth factor (VEGF), an emerging target for cancer chemotherapy. *Curr. Med. Chem. Anticancer Agents.* **3**, 95-117.

Smith, A. H., Ercumen, A., Yuan, Y., and Steinmaus, C. M. (2009). Increased lung cancer risks are similar whether arsenic is ingested or inhaled. *J. Expo. Sci. Environ. Epidemiol*. **19**, 343-348.

Smith, A. H., Lingas, E. O., and Rahman, M. (2000). Contamination of drinking-water by arsenic in Bangladesh: a public health emergency. *Bull. World Health Organ*. **78**, 1093-1103.

Soucy, N. V., Klei, L. R., Mayka, D. D., and Barchowsky, A. (2004). Signaling pathways for arsenic-stimulated vascular endothelial growth factor-a expression in primary vascular smooth muscle cells. *Chem. Res. Toxicol*. **17**, 555-563.

Takahashi, S., 2011. Vascular endothelial growth factor (VEGF), VEGF receptors and their inhibitors for antiangiogenic tumor therapy. *Biol. Pharm. Bull*. **34**, 1785-1788.

Tapio, S., and Grosche, B. (2006). Arsenic in the aetiology of cancer. *Mutat. Res.* **612**, 215-246.

Tischer, E., Mitchell, R., Hartman, T., Silva, M., Gospodarowicz, D., Fiddes, J. C., and Abraham, J. A. (1991). The human gene for vascular endothelial growth factor. Multiple protein forms are encoded through alternative exon splicing. *J. Biol. Chem.* **266**, 11947-11954.

Tseng, C. H., Chong, C. K., Chen, C. J., and Tai, T. Y. (1996). Dose-response relationship between peripheral vascular disease and ingested inorganic arsenic among residents in blackfoot disease endemic villages in Taiwan. *Atherosclerosis* **120**, 125-133.

Vahidnia, A., Romijn, F., van der Voet, G. B., and de Wolff, F. A. (2008). Arsenic-induced neurotoxicity in relation to toxicokinetics: Effects on sciatic nerve proteins. *Chem. Biol. Interact.* **176**, 188-195.

Verheul, H. M., Hoekman, K., Luykx-de Bakker, S., Eekman, C. A., Folman, C. C, Broxterman, H. J., and Pinedo, H. M. (1997). Platelet: transporter of vascular endothelial growth factor. *Clin. Cancer Res*. **3**, 2187-2190.

Webb, N. J., Myers, C. R., Watson, C. J., Bottomley, M. J., and Brenchley, P. E. (1998). Activated human neutrophils express vascular endothelial growth factor (VEGF). *Cytokine* **10**, 254-257.

Weis, S. M., and Cheresh, D. A. (2005). Pathophysiological consequences of VEGF-induced vascular permeability. *Nature* **437**, 497-504.

Xiao, Y. F., Liu, S. X., Wu, D. D., Chen, X., and Ren, L. F. (2006). Inhibitory effect of arsenic trioxide on angiogenesis and expression of vascular endothelial growth factor in gastric cancer. *World J. Gastroenterol.* **12**, 5780-5786.

Yu, M. J., Duan, Y., Liu, H., Zhang, X. M., and Li, H. M. (2006). Inhibited proliferation of B-lymphoma Raji cells and down-regulated expression of VEGF by arsenic trioxide. *Zhongguo Shi Yan Xue Ye Xue Za Zhi*. **14**, 704-707.

4.1 Objectives

Arsenic is a naturally occurring toxicant ubiquitously present in the earth crust. The general people are exposed to different (organic and inorganic) forms of arsenic through water, food, occupation and other environmental sources. Exposure to elevated levels of arsenic is a major health concern all over the world especially for Bangladesh where millions of people are currently at risk of arsenic poisoning. WHO has described arsenic toxicity in Bangladesh as one of the largest mass public health poisoning in the history of human civilization. Arsenic exposure is associated with a variety of cancers and vascular diseases. Cancer and vascular diseases are the major cause of arsenic-related mortality. There are several circulating inflammatory, adhesion, and angiogenic molecules that are associated with vascular diseases and cancer. Alteration of such molecules in blood can provide insight into the chronic diseases. This research has been designed to investigate the relationship between arsenic exposure and circulating molecules related to cancer and cardiovascular diseases (CVDs). Particularly this research has focused on 1) the investigation of the associations of arsenic exposure with C-reactive protein (CRP), intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1), the soluble markers of atherosclerosis that lead to the development of CVDs, and 2) the investigation of the associations of arsenic exposure with vascular endothelial growth factor (VEGF), a surrogate angiogenic marker involved in initiation and progression of variety of cancers and vascular diseases.

4.2 Summary of results

Chapter 2 represented the associations of arsenic exposure with plasma CRP, ICAM-1 and VCAM-1 related to atherosclerosis. These three proatherogenic molecules were significantly higher in arsenic-endemic individuals than those of non-endemic individuals. Then the dose-response relationships of these molecules were tested in non-endemic and tertile (low, medium and high) groups of arsenic-endemic subjects. Results showed that there were some general trends in changing ICAM-1 and VCAM-1 levels in the low, medium and high arsenic exposure groups and these changes were statistically significant in the higher (low, medium and high) exposure groups compared to non-endemic group. Plasma CRP showed good shape of dose-response relationships with higher exposure gradients and these differences were

statistically significant in the endemic tertile (low, medium and high) groups of water, hair and nail arsenic concentrations from the non-endemic group with few exceptions. Age, sex, BMI, smoking, hypertension, education, and monthly income were considered as covariates. The effects of these covariates on the observed associations of arsenic exposure with circulating molecules were evaluated. Interestingly, all the associations of arsenic exposure metrics with circulating molecules were significant even after adjusting for those covariates.

Chapter 3 represented the relationship of serum VEGF levels with arsenic exposure metrics. Significant positive correlations were observed with increasing concentrations of arsenic in water, hair and nails. The observed associations between arsenic exposure and serum VEGF were remain significant even after adjusting for relevant covariates such as age, sex, BMI, and smoking. Dose-response relationship of serum VEGF levels in tertile (low, medium and high) groups based on the three concentrations of arsenic in water, hair and nails were tested. Serum VEGF levels were found to be significantly higher in the medium and high groups compared to the low group in water arsenic concentrations. Similar dose-response relationship of serum VEGF levels were found in the medium and high exposure groups compared to the low groups in hair and nail arsenic. Further, dose-response relationship were tested among three groups (≤10 µg/L, 10.1-50 µg/L and >50 µg/L) based on the regulatory upper limit of arsenic in water set by WHO (10 µg/L) and Bangladesh Government (50 µg/L). It was observed that serum VEGF levels were significantly higher in >50 µg/L group than the ≤10 µg/L group. Interestingly, increased levels of serum VEGF levels were observed in the 10.1 – 50 µg/L group compared to the ≤10 µg/L group, although the difference was not statistically significant.

4.3 Strengths and limitations

4.3.1 Strengths

There were several unique features in the research.

First, the major strength of this dissertation was to show all the associations of CRP, ICAM-1, VCAM-1, and VEGF across the three kinds of exposure metrics (water, hair and nail arsenic concentrations). Therefore, the assessment of arsenic exposure by three kinds of exposure metrics and their correlations with CRP, ICAM-1, VAM-1, and VEGF might exclude the possibilities of miss classification.

Second, a good number study population with a wide variation of arsenic exposure levels that showed the dose-response relationship of arsenic exposure with CRP, ICAM-1, VAM-1, and VEGF levels.

4.3.2 Limitations

Despite major strengths, limitations can be attributed to some unmeasured or imprecisely measured potential confounding factors.

First, although we showed the association between the arsenic exposure and circulating CRP, ICAM-1, VCAM-1, and VEGF levels before and after adjusting for relevant covariates there might be some other factors such as co-exposure to other metals, insecticides or pesticides or individual variations that could influence the level of circulating molecules. If any accompanying metals or other contaminants could influence the observed associations, then they would also be expected to follow the same concentration gradients as arsenic in the drinking water, hair and nails. This is unlikely, but more extensive study of the other metals and their associations with CRP, ICAM-1, VAM-1, and VEGF levels are required in future.

Second, this study was designed to be cross sectional, hence causality was difficult to infer. A cohort based study is needed in future to verify the cause-effect relationship of arsenic exposure with CRP, ICAM-1, VAM-1, and VEGF levels.

Third, most of the study individuals were at the lower end of normal range of BMI. Thus, the results of the current study may not be generalizable to other study populations and the study needs to be replicated in other population.

4.4 Public health relevance

Arsenic is a potent environmental pollutant that has caused an environmental tragedy in some parts of the world especially in Bangladesh where tens to thousands of people have been affected because of the drinking of water contaminated by arsenic. Recent reports suggested that arsenic has entered the food chain including rice and vegetables. Since arsenic enters the food chain, the exposure to arsenic is unavoidable. Arsenic has been associated with several chronic diseases such as dermatitis, variety of cancers, CVDs, diabetes mellitus, liver and kidney dysfunctions. CVDs and cancer are the major causes of arsenic-related mortality in arsenic-endemic areas. CVDs are also the leading causes of death all over the world. Therefore, even small contribution of arsenic in the development of CVDs can cause a huge number of

excess deaths. Millions of people have already been affected by arsenic. Additional approximately 80 million are at risk of arsenic poisoning. This is the estimation in the case of Bangladesh. However, there are many other countries where arsenic toxicity has also taken as an endemic form. Therefore, this epidemiological research is completely relevant to the public health of those countries which are now under the threat of arsenic poisoning.

In this study, circulating molecules related to the development of CVDs and cancer have been measured. Circulating molecules are important in blood biochemistry to asses or predict the diseases condition. Increased levels of CRP, ICAM-1, VCAM-1, and VEGF may provide indication for the development of CVDs and cancer in future in arsenic-endemic individual. The endemic people, clinician, and health worker can take preventive measures against those diseases by monitoring those blood parameters. Further, the results of the research may be the valuable for the development of awareness among the arsenic-endemic people, policy makers, health workers and government about the adverse health effects of the chronic exposure to arsenic. Therefore, the results of this study may be important from policy perspective.

This research also stated that arsenic exposure dose-dependently associated with circulating CRP, ICAM-1, VCAM-1 and VEGF. All these molecules are implicated in the biochemical events of CVDs and cancer. Thus these results shed light on the mechanisms of those diseases. Understanding mechanism is critically important for the prevention and therapeutic intervention of the diseases. Therefore, objectives and findings of this study are very much relevant to the public health concern of Bangladesh and other arsenic-endemic countries of the world.

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Publications during the PhD period

5.1 Paper published from PhD research

i. **Rahman, M.**, Mamun, AA., Karim, MR., Islam, K., Amin, AA., Hossain, S., Hossain, MI., Saud, ZA., Noman, ASM., Miyataka, H., Himeno, S., and Hossain, K (2014). Associations of total arsenic in drinking water, hair and nails with serum vascular endothelial growth factor in arsenic-endemic individuals in Bangladesh**.** ***Chemosphere*** **120**, 336-342 (**Impact Factor: 3.499).**

ii. **Rahman, M*.,** Karim, MR*., Islam, K., Mamun, AA., Hossain, S., Hossain, E., Aziz, A., Yeasmin, F., Agarwal, S., Saud, ZA., Nikkon, F., Hossain, MM., Hossain, M., Mandal, A., Jenkins, OR., Haris, PI., Miyataka, H., Himeno, S., and Hossain, K. (2013). Increases in oxidized low-density lipoprotein and other inflammatory and adhesion molecules with a concomitant decrease in high-density lipoprotein in the individuals exposed to arsenic in Bangladesh**.** ***Toxicol. Sci.*** **135**(1), 17-25 (**Impact Factor: 4.478) [***Editorially high lighted article***].*equally contributed.

5.2 Paper published during the PhD period

i. Huda, N.**,** Hossain, S., **Rahman, M.**, Karim, MR., Islam, K., Mamun, AA., Hossain, MI., Mohanto, NC., Alam, S., Aktar, S., Arefin, A., Ali, N.,Salam, KA., Aziz, A., Saud, ZA., Miyataka, H., Himeno, S., and Hossain. (2014). Elevated levels of plasma uric acid and its relation to hypertension in arsenic-endemic human individuals in Bangladesh. *Toxicol. Appl. Pharmaco.* **doi**: 10.1016/j.taap.2014.09.011. (**Impact Factor: 3.63**). ***In Press***

ii. Hossain, E., Islam, K., Yeasmin, F., Karim, MR., **Rahman, M.**, Agarwal, S., Hossain, S., Aziz, A., Mamun, AA., Sheikh, A., Haque, A., Hossain, T., Hossain, M., Haris, PI., Ikemura, N., Inoue, K., Miyataka, H., Himeno, S., and Hossain, K. (2012). Elevated levels of plasma Big endothelin-1 and its relation to hypertension and skin lesions in individuals exposed to arsenic. ***Toxicol. Appli. Pharmacol.*** **259**, 187-194 (**Impact Factor: 3.63**).

iii. Islam, K., Haque, A., Karim, MR., Fajol, A., Hossain, E., Salam, KA., Ali, N., Saud, ZA., Rahman, M., **Rahman, M.**, Karim, MR., Sultana, P., Hossain, M., Akhand, AA., Miyataka, H., Himeno, H., and Hossain, K. (2011). Dose-response relationship between arsenic exposure and the serum enzymes for liver function tests in the individuals exposed to arsenic: a cross sectional study in Bangladesh**.** ***Environ. Health*** **10,** 64. (**Impact Factor: 2.71**).

iv. Reza, T., Aktar, S., Amin, HA., **Rahman, M.**, Arefin, A., Mohanto, NC., Alam, S., Mamun, AA., Habib, MA., Asafudullah, SM., Hossain, K., and Saud, ZA. (2014). In *vivo* analysis of toxic effect of hydrose indiscriminately used in food preparations in Bangladesh. *Asian Pac. J. Trop. Med.* **4**(11), 884-889.

v. Sheikh, A., Yeasmin, F., Agarwal, S., **Rahman, M.**, Islam, K., Hossain, E., Hossain, S., Karim, MR., Nikkon, F., Saud, ZA., and Hossain, K. (2014). Protective effects of Moringa oleifera Lam. leaves against arsenic-induced toxicity in mice. *Asian Pac. J. Trop. Med.* **4** (Suppl 1), S353-S358.

vi. **Rahman**, **M**., Saud, ZA., Hossain, E., Islam, K., Karim, MR., Hoque, MM., Yeasmin, T., Nikkon, F., Mandal, A.**,** and Hossain, K. (2012). The ameliorating effects of *Zingiber zerumbet L*inn. on sodium arsenite-induced changes of blood indices in experimental mice. ***Life Sci. Med. Res***, LSMR-41.

vii. Islam, MS., Alam, AHMK., Rahman, MAA., Ali, Y., Mamun, A., **Rahman, M.**, Hossain, AKMM., and Rashid, M. (2012). Effects of combination of antidiabetic agent and statin on alloxan-induced diabetes with cardiovascular diseases in rats. ***J. Sci. Rese.*** **4**(3), 709-720.

Annexure-I

Questionnaires to the patient/subject (personal information)

(**Confidential)**

Research Conducted by:

Department of Biochemistry and Molecular Biology, University of Rajshahi.

Subject/patient ID: ……..

Place of sample collection: …….

Date of data collection: ……..

PERSONAL INFORMATIONS

1. **Name of the subject**: ……
2. Father's /Husband's / Spouse's Name:
   …….....
3. Address: ……
4. Tel no: (if any): …….
5. Age: ……...
6. Sex: M F
7. Occupation: ……...
8. Body weight: …….. (in Kg)
9. Body height: ……. (in m) …….. (in ft)
10. Blood pressure: ……..
11. Marital status: i) Yes ii) No
12. Members in the family: i) One ii) Two iii) Three ii) Four
    iv) Five v) More
13. Socioeconomic conditions:
    Monthly income: i) Individual income ii) House hold income iii) others
    Monthly average income (In taka):

14. Education level: i) No ii) Primary iii) Secondary iv) Higher secondary v) Graduate level

15. Housing Status:

    a) Brick with concrete roof (Pakka)

    b) Brick with corrugated tin roof

    c) Mud with corrugated tin roof

    d) Straw (wall) with corrugated tin roof

    e) Others (thatched and tin wall with corrugated tin roof)

16. Sanitation: i) Yes ii) No

    If yes,

    i) Kacha (slab with straw or chat or bamboo wall) ii) Semi pakka iii) Pakka

17. Television: i) Yes ii) No

Information on arsenic exposure-related health problems

1. How many members in the family have been affected by arsenic?
……

2. Relationship of the arsenic-affected family members with the subject:

   i) Father ii) Mother iii) Husband iv) Brother v) Sister vi) Son

   vii) Daughter or viii) Wife

   ix) Others (specify)……

3. What are the age and sex of children:

   i) 1st child……. sex: M F

   ii) 2nd child ……sex: M F

   iii) 3rd child…….sex: M F

4. From when symptoms of arsenicosis have been developed in the child?
……..……

5. How long is he/she residing in the study area?

   i) 1 year ii) 2 year iii) 5 years iv) More (specify the year)….....

6. Drinking water sources: i) Tube-Well ii) Kua

   Is the source of drinking water contaminated?

   i) Yes ii) No iii) Not yet confirmed.

   If yes, from when he/she came to know?
   ..……Years

   Has the drinking water source been checked for arsenic contamination?

   i) Yes ii) No iii) unknown

   Has the tube well marked by red color?

   i) Yes ii) No

7. How long did he/she drink water from that source? …… years.

8. Major symptoms of arsenicosis
(Specify the symptoms) ……..……

   a) Skin (specify the symptoms):

      i) Melanosis ii) Hyperkeratosis iii) Both

   b) Respiratory complications (specify the symptoms):

i) Asthma ii) COPD iii) DPLD iv) Cough

v) Haemoptysis v) SOB vi) Chest pain

c) Urinary related problems (specify the symptoms):...……

d) Eye related problem (specify the symptoms):……

e) Diabetes: .....……

f) Neural problem (specify the symptoms):……

g) Taste (decrease, increase or unknown):……

h) Cardiovascular system (specify the symptoms):

i) IHD ii) Hypertension iii) Heart failure

i) Hair loss: .....……

j) Allergy (specify the symptoms):.……

k) Hearing Problem: .....……

l) Others problems: …….....……

9. a) Has the subject already gone to the physician? i) Yes ii) No

If yes, for what problem (Specify it)? ----------

b) Did the physician give you any medicine? i) Yes ii) No iii) Unknown

c) What types of medicine (specify the drugs).....

d) Did the physician give you any medicine for the treatment of arsenicosis? i) Yes ii) No

e) What types of medicine (specify the drugs) ……

10. Has any agencies/person checked arsenic levels in the food/vegetables/fishes which are consumed by subject? i) Yes ii) No

(If yes, please specify the types of food which contain high level of arsenic)

……

Thanks for your participation and cooperation.

Name & Signature of the Investigator (s):

Date:

Additional comments: