EFFECT OF DIET, MICRONUTRIENTS ANDBIOMETHYLATION ON CHRONIC ARSENIC TOXICITY – A HEALTH SERVICES RESEARCH PERSPECTIVE

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COMMITTEE IN CHARGE OF CANDIDACY

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ABSTRACT

Environmental epidemiological studies can contribute to the development of explanatory and predictive models of health care access and utilization. The purpose of this dissertation is to develop a multidsiciplinary perspective to integrate health services research with environmental epidemiology. The context of this study was Arsenic toxicity and related skin lesions in India.

Exposure to inorganic Arsenic in groundwater occurs worldwide but is particularly serious in the Gangetic delta in the Indian subcontinent. Here, nearly 140 million people are exposed to inorganic arsenic exceeding 50 micrograms/liter. While exposure to high concentrations of inorganic Arsenic is commonly associated with arsenic caused skin lesions, other associated clinical disorders include cancers of skin, liver, lung, urinary bladder, and diabetes and bronchiectasis. It is believed that improved nutritional status through diet and micronutrient supplementation may protect against Arsenic toxicity and increased methylation through MMA generation may be associated with increased toxicity. It is unknown to what extent diet, micronutrients influence arsenic caused skin lesions and methylation, and in turn how methylation influences arsenic caused skin lesions or other diseases. From the perspective of health services research, this information is relevant for identifying macro and micropathways of Arsenic caused health effects from a health services research perspective. This also contributes to the explanatory models of health services utilization and access to care with environmental parameters as key variables.

A cross sectional survey was conducted on 7683 individuals in West Bengal, India, between 1995-1996. Nested within the survey was a case control study of 405 individuals (192 cases with skin lesions and 213 controls) on the association between diet, micronutrients and skin lesions. Information on diet was obtained with a food frequency questionnaire, blood and urine samples were collected from each participant for measurement of micronutrients, and urinary metabolites of inorganic Arsenic. A series of multivariate models were used to study the mutual association

between diet, micronutrients, methylation and skin lesions in this population in a way to delineate the micro and macropathways related to clinical correlates and methylation related to arsenic toxicity.

Results of multivariate analyses suggested that high dietary intake of animal protein (OR: , 95% CI:), dietary phosphorus (OR: , 95% CI:), Calcium (OR: , 95% CI:), and vitamin C (OR: , 95% CI:) were associated with reduced risk of skin lesions. Asociation between methylation and skin lesions suggested that individuals with low InAs% (OR: , 95% CI:) and high DMA% (OR: , 95% CI:) were associated with higher risk of skin lesions. Finally, analysis of diet, micronutrients and arsenic methylation suggested that dietary animal protein, serum selenium, and plasma folate were associated with high MMA%, a factor known to be associated with cancerous health effects of arsenic exposure .

These findings provide supportive evidence of the need for an interdisciplinary approach to model building in health services access and utilization. Environmental epidemiology is integral to the explanatory and predictive models of health services delivery and utilization.

(350 words needed, now 527 words)

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DEDICATION

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CHAPTER 1: STATEMENT OF THE PROBLEM

Abstract

Health states are influenced by complex interactions between environment and genetic predisposition, and therefore health services, organized around provision of preventive services, health promotion and curative services, need to take into account the role environment plays in influencing health care access and utilization. However, environment also interacts in complex ways with biomedical and clinical factors involved in health care access and utilization. The purpose of this dissertation is to provide an insight into model building in health care access and utilization with contribution from environmental epidemiology. The complex global problem of arsenic toxicity (specifically in the context of Indian subcontinent) will be considered to develop a framework of the process incorporating environmental epidemiological studies into health services research

Therefore, in this introductory chaper, the purpose is to provide an introduction to the inter-relationship between environmental epidemiology and health services resarch. This will be done by discussing the epidemiology of inorganic Arsenic toxicity in the Indian subcontinent in particular in the context of the global problem, outline the key mechanisms of arsenic toxicity, in particular the biomethylaiton of arsenic, and address how diet and micronutrient supplementation can potentially alter the course of the conditions and why diet and micronutrients are essential to contain the arsenic epidemic and how these issues are associated with health services organization and research. Second, based on the background research, the key research questions that connect arsenic epidemiology, methylation of arsenic and mititgation of arsenic toxicity will be discussed. Finally, in this chapter an outline of the key issues and questions are provided, and a framework of presentation of the topic and subsequent chapters.

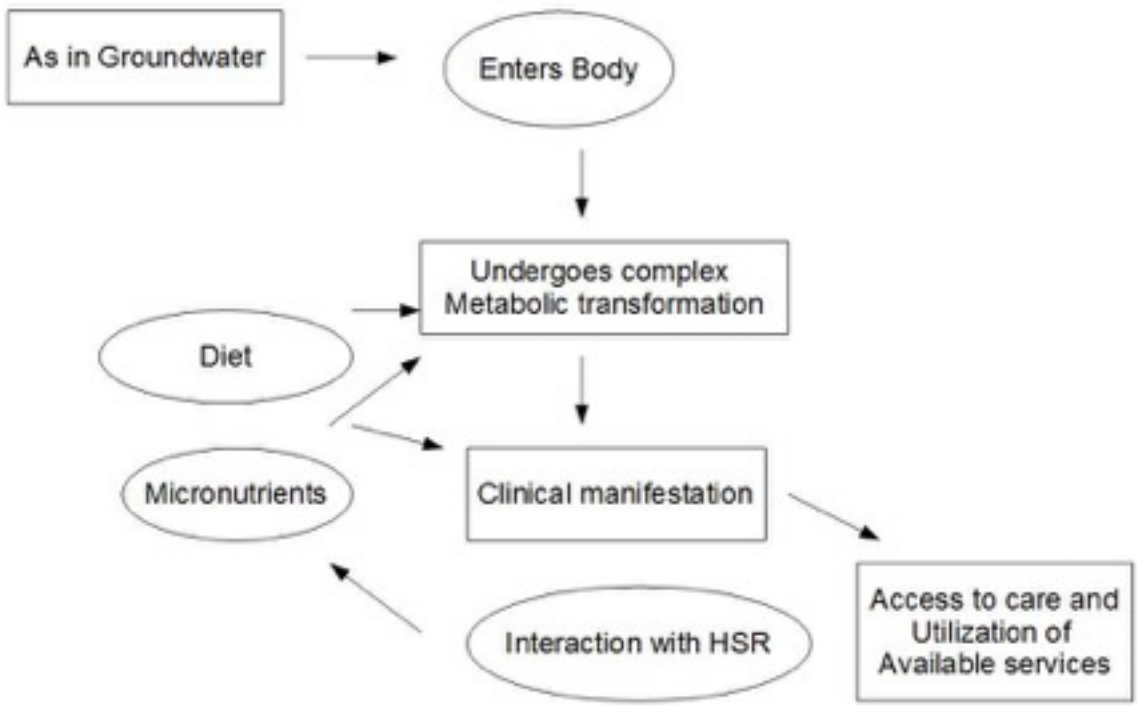
Exposure to inorganic Arsenic occurs worldwide and is particularly serious in the Ganges delta. About 140 million people are exposed to high concentrations of inorganic Arsenic through groundwater supply. Exposure to inorganic Arsenic is associated with development of characteristic skin lesions, and other malignant and non-malignant conditions including bronchiectasis, and cancers of lung, liver, urinary bladder. Following entry into body, inorganic Arsenic undergoes a series of reduction and methylaiton reactions. As a result of these transformations, inorganic Arsenic is converted to monomethyl arsonous acid (MMA) and dimethyl arisinic acid (DMA) and excreted in urine. However, because of these stages of biotransformation, in presence of heavy load of inorganic Arsenic, deficiencies of micronutrients occur and other biological processses such as DNA formation and maintenance that are in turn dependent on biomethylation get affected. These processes result in pathological processes lead to arsenic-caused malignancy and other health effects. In addition, while there is no effective pharmacological treatment for reversal of Arsenic toxicity, it is believed that diet rich in protein and micronutrient supplementation may protect against or minimize the impact of Arsenic-caused diseases in population heavily exposed to Arsenic. However, it is also not known to what extent diet, and micronutrients are related to methylation.

Finally, clinical expressions of different forms of skin lesions, skin cancers, diabetes, other diseases, and malignant conditions define the interface between environmental health and health care organization. Thus, environmentally mediated diseases such as skin lesions, diseases of lung, and urinary bladder cancer in the Arsenic-exposed community change the patterns of access to care and challenge in the organization of preventive and palliative health care services. Therefore, studies in populations that are heavily exposed to environmental agents such as Arsenic provide opportunities to examine and modify explanatory and predictive models of health services organization, utilization, and access.

In this dissertation, these four concepts have been examined using results from a nested case control study within a large cross-sectional survey in an Arsenic-exposed population in the North 24-parganas district in India. The following questions are addressed in the subsequent chapters.

1. What is the association between diet, micronutrients (nutritional factors) and arsenic-caused skin lesions?
2. What is the association between methylation and arsenic-caused skin lesions
3. What is the association between nutritional factors and arsenic methylation?
4. How does these associations inform the process of health services research and health services utilization and access?

Figure 1. Inter-relationship between nutritional factors, biomethylation, clinical outcomes, and



interaction with health services utilization and access

The inter-relationships between exposure to inorganic Arsenic, the metabolic transformation of inorgnaic arsenic through the process of methylation, the resulting clinical manifestation (skin lesions, skin cancers, diabetes, and cancers of other organs), diet and micronutrient supplementation are complex but important to understand for the mitigation of Arsenic toxicity related deaths and disability from a public health perspective. A complex set of social, and economic causes resulted in people's access to arsenic.

The organization of the chapters for this dissertation are as follows. In this chapter the epidemiology of Arsenic toxicity in South Asia and its relationship to the clinical outcomes and helath care access and scope of the dissertation is briefly described. In addition, in this chapter, the mechanism of action of Arsenic (methylation), and discuss the evidence on the association of methylation and arsenic caused health effects. In chapter 2 will be discussed the methods of the survey, and the organization of the case control study design. In chapter 3 will be discussed the association between diet, micronutrients, and arsenic-caused skin lesions. Chapter 4 will discuss the association between methylation and arsenic-caused skin lesions. Chapter 5 will discuss the association between nutritional factors and arsenic methylation. Finally, in chapter 6 will be discussed the implications of these findings for health services research by examining the behavioural model of health services access and utilization of care and life course health development perspective of access and utilization of care. In particular, it will be examined how incorporation of environmental epidemiological data can address the “silo” based approaches that do not address the theoretical propositions of each model.

Epidemiology of Arsenic toxicity

[Figure 1 goes here]

Figure 2: Distribution of exposure to inorganic Arsenic throughout the

world

Table 1. Prevalence of Arsenic-caused health effects

Health Effect	Prevalence	Population	Author (year)
Arsenic-caused skin lesions	714 confirmed cases of out of 14, 828 individuals in a cross sectional survey (melanosis and keratosis combined)	Bangladesh	Ahsan (2006)
Arsenic-caused non-cancerous skin lesions (keratosisi and melanosis)		India	Guha Mazumder ( )
Basal Cell Carcinoma		Americans of European origin, Hungary, South America	
Bowen's Disease		Asian countries, Thailand, Taiwan	
Chronic bronchitis			
Bronchiectasis			
Lung Cancer			
Liver cancer			
Cancer of urinary bladder			
Diabetes			

[Figure depicting a number of Arsenic-caused skin lesions]

Figure 2: Arsenic caused skin lesions and other clinical manifestations

Arsenic methylation and mechanism of action

[Figure depicting As --> As III --> MMA --> DMA]

Figure 3: Steps of inorganic Arsenic methylation

Statement of the problem and hypotheses

What is the association between nutritional factors and Arsenic caused skin lesions?

What is the association between methylation and Arsenic caused skin lesions?

What is the association between nutritional factors and methylation of Arsenic?

How do environmental epidemiology of Arsenic inform the process of health services research

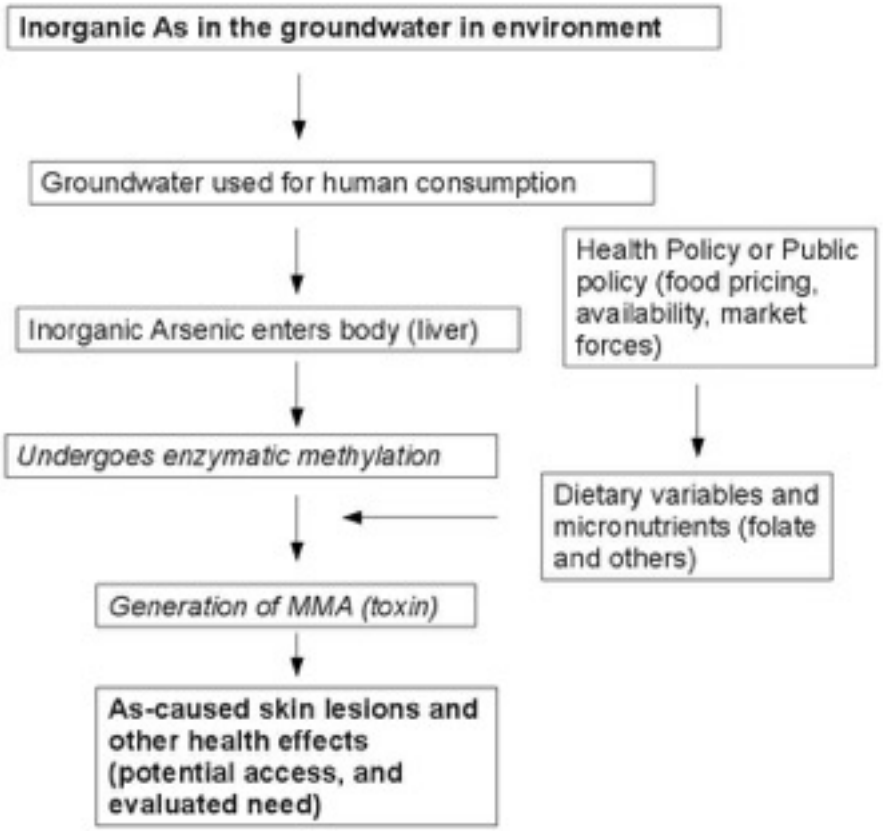


Figure 4: Interface between arsenic toxicity and health policy

Organization of the subsequent chapters

The methods and the study design for the survey and data analysis are outlined in Chapter 2. The data analysis, the results, and the discussion of the impact and health services implications for the association between nutritional factors and arsenic caused skin lesions are described in Chapter 3. In Chapter 4, the plans of data analysis, results, and implications of the results on the association between methylation and skin lesions are described. In Chapter 5, the plans of data analysis, results and implications of the findings with respect to health services research and access are outlined for the association between nutritional factors and methylation. Finally, in Chapter 6, the relationships between health services research, access and utilization of care and environmental epidemiology are discussed.

CHAPTER 2: POPULATION AND METHODS

Abstract

The purpose of this chapter is to provide the methodology of the cross sectional survey, and the organization of the case control study. This chapter provides an outline of selection of the population, organization of the cross sectional survey, the process of food frequency questionnaire based data collection, collection of urine samples and blood samples for further analysis at the University of Washington laboratory of Professor David Kallman, development of the nutritional data analysis software programme, and analysis of the data.

Population under study

Steps of the cross sectional survey

Steps of the nutritional data analysis

Steps of the blood nutritional factor analysis

Steps of the urine analysis

Statistical data analysis

CHAPTER 3: NUTRITIONAL FACTORS AND SKIN LESIONS

Abstract

Exposure to inorganic arsenic is associated with development of characteristic skin lesions (alternating dark and light pigmentation, keratosis or thickening of skin, and other lesions). It is believed that diet and micronutrient supplementation can result in protective effects against development of arsenic caused skin lesions and other clinical manifestations of arsenic toxicity.

A nested case control study was conducted within a larger population based cross sectional survey of 7394 individuals. Dietary information were obtained using a food frequency questionnaire; blood (plasma and serum) micronutrients were collected at the same time as conducting the diet survey and urine specimens were collected at the time of conducting the survey. The blood and urinary assays for micronutrients were conducted at the University of Washington laboratory. The clinical information on the participants were obtained by trained physicians. The data were analyzed using a matched case control study and logistic regression models.

Arsenic toxicity in West Bengal and its impact on Health Services

Arindam Basu

Sept 7, 2007

Abstract

Worldwide, millions of people who are exposed to inorganic arsenic present with skin lesions, and cancers of skin, urinary bladder, liver, and lungs, among other diseases. In South Asia, in the states of West Bengal of India and the adjoining districts of Bangladesh alone, about 130 million people are exposed to inorganic arsenic exceeding 50 micrograms per liter in their drinking water, about 5 times the maximum contaminant level set by the United States Environmental Protection Agency and the WHO. The emergent burden of diseases will likely to impact patterns of healthcare utilizations for dermatological and oncological services for these countries. Epidemiological studies suggest that diet and select micronutrients have limited roles in the pathogenesis of arsenic poisoning, and that in vivo methylation may enhance toxicity or increase susceptibility to arsenic poisoning. In this dissertation, the evidence from available studies on the inter-relationships between diet, methylation and arsenic-caused skin lesions will be examined in combination with primary studies conducted in an arsenic-exposed population in West Bengal, India. Based on the findings of these studies, this dissertation will examine an existing model of health care utilization profiles for people living in high exposure areas. It is hypothesized that in addition to health care seeking behavior, health care utilization patterns are also functions of physical environment. Finally, this dissertation will argue for an interdisciplinary approach to better comprehend patterns of healthcare utilizations, breaking away from the current "silo" approach of non-integrating disciplinary work in explaining health care utilization profiles.

1 Overall Problem

Worldwide, environmental exposure to inorganic arsenic accounts for considerable morbidity and mortality [2]. Continuous exposure to inorganic arsenic over 5 to 10 years eventually leads to skin

discolorations and thickened lesions (hyperpigmenation and keratosis), peripheral neuropathies, chronic respiratory diseaes (chronic bronchitis and bronchiectasis), diabetes mellitus, and cancers of skin, urinary bladder, liver, renal failure and lungs [20]. The International Agency for Research on Cancer has stated that there exists sufficient evidence in humans that arsenic in drinking water causes cancers of lung, urinary bladder, and skin [1].

In developing countries, drinking water is the most common route of exposure to arsenic; others include food, and air. In 2001, the United States Environmental Protection Agency revised the maximum contaminant level of arsenic in groundwater and recommended a maximum contaminant level of exposure to inorganic arsenic at 10 micrograms per liter, based on the risk estimate of 1 in 300 for arsenic levels at 10 micrograms per liter [14]. At exposure levels of 50 mcg/L – a maximum contaminant level still adopted by India and Bangladesh are 5 times the current recommended maximum contaminant level. Millions of people worldwide are exposed to high concentrations of arsenic. In South Asia alone, between Bangladesh and the adjoining West Bengal state of India, about 130 million people are exposed to inorganic arsenic through their groundwater supply exceeding 50 micrograms/L, and are at risk of developing long term consequences of arsenic exposure [16, 11]. Large scale exposure to inorganic arsenic in drinking water began in the 1960s in South Asia, when shallow alluvial aquifers of the Bengal delta were tapped to obtain groundwater. This was primarily done under guidance from International Agencies in order to reduce the burdens of diarrheal diseases brought about by consumption of coliform bacteria-contaminated surface water from lakes and tanks and to supply water for irrigation. In the sixties, in Bangladesh alone, the infant mortality rate from diarrheal diseases were about 14 per 100, making it to be the leading killer disease. Installation of tubewells resulted in about 44% reduction in infant mortality from diarrheal diseases in Bangladesh. Additionally, installation of tubewells also made light of the workload of the womenfolk to fetch water from long distances and provided an ownership of having their source of drinking water in their own homes [4]. These twin effects sustained the

acceptability of tubewells as a source of drinking water in a large number of households across South Asia. Arsenic-caused skin lesions emerged as public health problem in South Asia in the 1980s. Since 1983, when the first cluster of 16 cases of arsenic-caused skin lesions were detected at Calcutta's School of Tropical Medicine by Professor Kshitish Chandra Saha, thousands of cases of arsenic-caused skin lesions and other arsenic-caused diseases have been diagnosed in West Bengal and Bangladesh. The large number of patients with arsenic-caused health effects as a result of exposure to high concentrations of arsenic in their drinking water has been compared to environmental disasters in Bhopal and Ukraine and described as the worst environmental disaster in the twentieth century [15, 18].

Inorganic arsenic remains in groundwater sources largely in As(V). Once it gains entry to human body through drinking water, inorganic arsenic undergoes series of biotransformations in liver sequential stages of alternative reduction and enzyme mediated biomethylation. These metabolic stages result in arsenic interacting with body tissues and altered methylation patterns of DNA and cellular transport mechanisms leading to its pathogenesis. Following entry to human body, As(V) is first reduced to As(III) and then sequentially methylated to monomethyl arsonous and arsonic acids (MMA(III) and MMA(III)) and dimethyl arsinic acids (DMA(III) and DMA(III)). It was earlier believed that methylation was a detoxification process; following isolation of MMA(III), studies with human tissues and animal experiments suggest that MMA(III) can be more toxic compared to Arsenic (III) and As(V), and consequently, methylation may not be entirely detoxifying, and in fact,can be harmful. Methylation of arsenic is incomplete and ingested inorganic arsenic is excreted as 10-20% as inorganic arsenic, 10-15% as monomethyl arsenic (MMA), and 60-75% as dimethyl arsenic (DMA) [10]

Data from the dose-response studies conducted in Taiwan in 1989 and 1992 on the association between arsenic exposure and arsenic-caused skin cancers suggested a strong dose response effect [20]. Engel and colleagues suggested that low intakes of micronutrients in Taiwan population

may have made them more susceptible to arsenic-caused skin cancers [12]. Results from a matched case control studies among Atacameno people in Chile suggested that despite good nutritional status of these population, the prevalence of arsenic caused skin lesions in this population as a result of exposure to arsenic laced drinking water were comparable to that in comparable regions of the world [17]. A large nested case control study within a larger cross sectional survey conducted in an arsenic-endemic population in West Bengal, India reported that people who were in lowest quintiles of dietary intakes of protein were likely to be susceptible to arsenic-caused skin lesions, and another study on the blood levels of micronutrients and arsenic-caused skin lesions failed to find associations between selenium methionine, and beta-carotenes and arsenic-caused skin lesions [12, 6]. No data is available on the impact of diet, together with micronutrients on arsenic-caused skin lesions.

Environmental epidemiological studies constitute the evidence base for key health care policies aimed at mitigation of the arsenic toxicity in arsenic-endemic populations. For instance, animal- and human tissue-based experiments suggest that inorganic arsenic and selenium antagonize each other, and that selenium deficiency may increase susceptibility to arsenic induced cancers of liver [7, 9, 19]. While the "perceived" efficacy of selenium supplementation on theoretical and biological grounds might be justified to initiate an expensive public health program of population based selenium supplementation in Bangladesh at great expense, the outcome of at least one placebo controlled randomized trial of selenium supplementation had low efficiency. In one placebo controlled double blind randomized controlled trial of selenium supplementation, either alone or in combination with Vitamin E supplementation, the investigators found that selenium supplementation had modest improvement in arsenic-causes skin lesions that were not statistically significant [Error: Reference source not found]. it failed to mitigate the arsenic toxicity profiles of the Bangladeshi population to whom it was targeted. To understand the gap between efficacy and efficiency of mitigation programs driven by solely environmental epidemiological findings and

limited understanding of biology of arsenic toxicity bereft of the context in which toxicity and the health care delivery response occurs deserves a fuller explanation.

Interaction between emergence of environmental health problems and health care utilization and access to care is a dynamic process. In the context of arsenic toxicity, groundwater residing at 10-80 meter depth was tapped in 1960s, in order to find a solution to increased infant mortality and ill health related to drinking surface water that was contaminated with coliform bacteria. Before initiation of tubewells in Bangladesh, the work of fetching water from long distances was an additional task for the womenfolk of the households, and use of tubewells soon became widespread as people invested their resources in the technology to bore tubewells closer to their homes, often in their backyards. The net result was now there was an ownership of drinking water and this was prized [5]. However, at the time of sinking these tubewells, the water was not tested for the presence of inorganic arsenic in them. In 1983, the first cases of arsenic toxicity emerged in West Bengal, India, when dermatologists started seeing more cases of palmar and plantar hyperkeratoses [18]. Thus, a sudden increment in the utilization of dermatological services was associated with further testing of their drinking water sources which revealed high concentrations of arsenic in the groundwater. At the estimated prevalence of 20 percent, and an exposed population of 42 million people in West Bengal who are exposed to high arsenic in drinking water, over the next 5-10 years, about 8 million people would be likely to use not only dermatological services but also be additional burdens to urological services (because of the association between arsenic and urinary bladder cancer), respiratory care (association between arsenic and chronic respiratory diseases, lung cancer, and bronchiectasis), and liver diseases.

Traditional explanatory models of health services research and health care utilization focus on behavioral constructs of health care utlization patterns. Ronald Andersen's Behavioral Model, developed initially in the 1960s and later expanded with 4 iterations, has been widely used for explaining and predicting health care utlization pattern for families and individuals. Briefly, in this

model, health care utilization has been explained by three inter-related factors: predisposing factors, for instance, a person's demographic and socioeconomic factors which also include physical environment, enabling factors (including social situations, financial status, existing health policies) and need factors (evaluated need factors that which is determined by the physicians or realized need where the patient actually accesses the healthcare system and complies). Study by Aday and colleagues suggest that provider-related and contextual variables explain the utilization patterns of heathcare by about 20 percent. It may be argued that external physical environmental factors can also explain variations in healthcare utilization patterns, as can be shown by the case with arsenic toxicity in South Asia [3, 13].

The purpose of this dissertation is to describe the inter-relationship among diet, micronutrients, arsenic methylation, and arsenic-caused skin lesions in an arsenic-exposed population in the state of West Bengal in India, and bridge the translational gap between environmental epidemiological studies and the traditional models of health services research that focus on behavioral components of health care utilization patterns, putting less emphasis on the nascent variables of environmental health related agent provocateurs that impact how people access and utilize existing health care delivery systems. In building this argument, we have laid out the chapters as follows. This first chapter will discuss a brief history of the arsenic toxicity in South Asia, followed by description of the health impacts of arsenic exposure. In describing the health impacts of arsenic exposure, we shall limit ourselves to the arsenic-caused skin lesions, since these develop after 5-10 years of exposure to inorganic arsenic and are specific to arsenic toxicity. We shall conclude this first chapter with a discussion on the interface with health services research issues, specifically how arsenic toxicity, as a talking point, builds on the health behavioral model proposed by Andersen and how potentially, the scope of the Andersen model can be expanded with data from enviromental epidemiologic studies. Chapter 2 will describe the association between diet, micronutrients, and arsenic-caused skin lesions. Chapter 3 will describe the association between methylation patterns

and arsenic-caused skin lesions. Chapter 4 will describe the association between diet, micronutrients, and patterns of arsenic methylation. Finally, chapter 5 will begin with a restatementn of the overall goal, summarize the study findings, discuss the limitations of these studies in applying them to the health services research framework, and finally implications of these studies for formulating health policies and future directions that research might take to integrate environmental epidemiologic studies and health services research paradigms.

2 History of Arsenic Toxicity in South Asia

In the nineteen sixties, demand for water for drinking and irrigation increased in India and Bangladesh because of increased population and agricultural activities. At that time, surface water from lakes, ponds and rivers were the only sources of water for drinking and agricultural purposes. Surface water had high coliform bacterial load and consumption of surface water resulted in high prevalence of diarrheal diseases, particularly in young children, with prevalence was about 140 per 1000 population [5]. To address the need of increased demand for water and increasing burden of diarrheal diseases among children, UNICEF (United Nations International Children's Emergency Fund) and other agencies advised the Government of India to sink of tubewells at depths ranging between 20 and 60 meters in order to tap groundwater. During that time, water was not tested for the presence of inorganic arsenic. Following sinking of tubewells, mortality rates due to diarrhea fell in BD by 44% (UN Report, 1999). At the time of sinking tubewells, presence of arsenic in groundwater was not tested. Presence of arsenic in groundwater went largely undetected for a long time and therefore no mitigation efforts were instituted because inorganic arsenic in groundwater does not impart any color or smell to the water consumed and therefore invisible. In 1983, Dr. Kshitish Chandra Saha, a dermatologist in Calcutta's School of Tropical Medicine and Hygiene first reported a case series of patients he had examined for the presence of arsenic-caused skin lesions. The first cases of arsenic-caused skin diseases were reported in 16 individuals from one village in 1983. In 1986, following a newspaper report about a patient from the villages in South 24-Parganas

who attended the clinic of Professor KC Saha, the Government of West Bengal initiated investigations into the extent of arsenic toxicity in West Bengal [16].

By 1987, 6 districts in West Bengal was declared to contain arsenic in groundwater and xx districts in Bangladesh was found to contain arsenic in groundwater above the levels of 50 micrograms/ L. In 1995-1996, Dr DN Guha Mazumder initiated the first large scale cross sectional survey in West Bengal to study the prevalence of Arsenic toxicity in West Bengal. In 2001, the United States Environmental Protection Agency revised their recommendations for the maximum contaminant level for arsenic from 50 micrograms/L to 10 micrograms/L. However, Government of India and Bangladesh maintained the maximum contaminant levels at 50 micrograms/L. This was justified in view of the large number of people who were exposed, the costs to be invested in technology to reduce the well water concentration to 10 micrograms/L and to maintain the impact of the mitigation efforts like dug well programs [8].

3 Descriptive Epidemiology

3.1 Prevalence of arsenic caused skin lesions

In the West Bengal state of India, about 42.7 million people spread across 39,000 square kilometer area in 9 districts were exposed to arsenic in groundwater exceeding 50 micrograms per liter. with a total population of 68 million in West Bengal, this indicates that about 63% of the total populaiton in West Bengal is exposed to arsenic in drinking water exceeding 50 micrograms/L and therefore at risk. In Bangladesh, about 79.9 million people in 42 districts, spread across 150,000 square kilometer are exposed to arsenic levels exceeding 50 micrograms per liter. Bangladesh has 64 districts in all, with about 120 million population. Thus, in Bangladesh, about 66.7% of the population of Bangladesh are exposed to arsenic in groundwater exceeding 50 micrograms per liter, exposing them to arsenic caused skin lesions and other clinical effects of arsenic exposure. In Bangladesh, a total of 11 million tubewells are known to have arsenic concentrations of 50

micrograms/liter or higher. About 97% of the wells in the South and Southwestern parts of Bangladesh are known to contain arsenic exceeding 50 mcg/L.

Nine cross-sectional surveys in West Bengal and Bangladesh were identified that aimed to study the prevalence of arsenic-caused skin lesions among the population in different villages or districts of the two regions. Data were abstracted from these surveys and prevalence of arsenic caused skin lesions were calculated for the entire population they surveyed. Based on their study estimates, the ranges of arsenic-caused skin lesions were found to vary between 3 per 1000 for a large cross sectional survey of 166,934 individuals in the xx district of Bangladesh to 290 per 1000 population in the yy district of Bangladesh. For West Bengal, the prevalence ranged between 46.9 per 1000 for hyperpigmentation (combined for men and women for all ages), to 190.4 per 1000. In general, prevalence were found to be high for those studies that had non-random sampling (based on volunteer based reporting). The estimated median prevalence of arsenic-caused skin lesions among arsenic-endemic areas of West Bengal and Bangladesh would be about 68.9 per 1000 population.

Increased age was associated with increased risk of arsenic-caused skin lesions, and men had higher rates of arsenic-caused skin lesions than women. Association between socioeconomic status and arsenic-caused skin lesions were inconclusive. In one large population based cross sectional survey in Bangladesh, people who had higher socioeconomic status (measured by high total family income) had higher risk of arsenic caused skin cancers in multivariat models. The authors commented that this was because of high private tubewell ownership and consequently a function of high intake of arsenic-laced drinking water.

Low dietary intakes of specific dietary items and specific micronutrients are associated with increased risk of arsenic-caused skin lesions in West Bengal and Bangladesh. However, the combined effects of low dietary intakes of specific dietary items and micronutrients taken together on arsenic-caused skin lesions is unknown. This problem has been addressed in this dissertation.

Increased methylation is associated with arsenic-caused health effects, including arsenic-caused skin cancers, and cancers of urinary bladder. One cross-sectional survey in Taiwan noted that increased methylation and output of MMA in urine was associated with increased risk of arsenic-caused skin pigmentation.

4 Interface with Health services research

Environmental arsenic poisoning accounts for considerable morbidity and mortality worldwide in general, and in particular, for Bangladesh and the West Bengal state of India. As we identified before, about 63% of the total population of West Bengal (N = 42.7 million) and 67% of the total population of Bangladesh (N = 79.9 million) are exposed to arsenic in their groundwater exceeding 50 micrograms per liter. Assuming an average prevalence of 6.9 % individuals exposed to arsenic levels exceeding 50 micrograms per liter would develop arsenic-caused skin lesions, it is estimated that in West Bengal, about 2.95 million people would develop skin lesions in 5 –10 years following exposure. Similarly, for Bangladesh, an estimated 5 million people would develop arsenic-caused skin lesions. In addition, assuming 1 in 300 individuals would develop skin cancers or internal cancers resulting from exposure to arsenic in groundwater, an estimated 142,333 people would develop cancers resulting from arsenic exposure in West Bengal and 266.333 people in Bangladesh will develop arsenic-caused cancers of skin, urinar bladder, and lungs over the next 20 years. These figures suggest that utilization for oncological and dermatological services are likely to be considerably higher in areas of high arsenic-endemicity compared to residents in geographical areas where arsenic exposure is not a major environmental health problem.

Traditionally, health services utilization have focused on health behavior pertinent to utilization of health services. A popular theory of health services utilization was proposed by Ronald Andersen in 1960s and was subsequently modified by Andersen, Aday and others through four iterations.

Briefly, Andersen reviewed the history and emergence of a model explaining health care

utilization in four phases. The first iteration was his original theory in 1960s where he posited that health care utilization profiles for families could be explained by invoking specific predisposing, enabling and need based factors. This was modified in the 70s and eighties where additional dimensions of health status and health outcomes were added to the model. In the third iteration of the model, that was prevalent in the last decade of the twentieth century the focus was on health status and health outcomes as well as on personal health behavior. He added a new category to the access variables in addition to the original groupings in terms of potential accesss that the patients had resources available and the society provided them opportunities to access the health care services, realized access to what extent the services were utilized, the equitability of accesses whether demographic and need variables determined the access to healthcare issues when that would be termed as equitable access and when variables such as resources and health beliefs wereexplanatory variables for healthcare access, then that would be defined as inequitable access. In the third iteration, Andersen added concepts of effective and efficient accesses. Effective access was defined s access that improved health status or satisfaction of the clients who accessed healthcare in this phase. Efficient access was whether, in comparison to the resources used or utilized, the health status or satisfaction with healthcare increased substantially. Andersen argued about the more mutable a factor was, the more it could contribute to explain the variability associated with health care utilization patterns. For instance, demograhic and social factors were not mutable at all, while health policy related variables that included clinical guidelines etc that tended to vary with improved research and greater access to information. In the fourth iteration of his model, he included feedback loops to the model where he stated that outcomes would influence thedeterminants of the utilization pattern and consumer satisfaction and other system variables. Notable in this descriptions of health care utilization models were the absence of the impact of physical environment. Being included as immutable predisposing factors, the importance of physican environmental variables were perceived less important in comparison to other factors.

Andersen's model was criticized and modified by Phillips and colleagues where they stated that while Andersen's model laid emphasis on the individual behavior for explaining health care utilization, it did not sufficiently discussed issues related to either environmental or provider-related variables. By environmental, Phillips indicated variables that related to health care delivery system characteristics like policy, resources, etc, (b) external environmental variables mainly relating to financial and economic and social environment, rather than physical environment, and community level enabling factors.

Experience in South Asia on the emerging arsenic toxicity and the potential impact of arsenic toxicity on health care utilization patterns suggest a review of the existing models of health care utilization and the need for factoring in external environmental variables as important determinants of healthcare utilization patterns to explain the variability in the healthcare utilizations in specific areas where environmental health related issues are important contributors to illness profiles in these areas.

This dissertation will provide an evidence base for health policy aimed to reduce the burden of diseases due to arsenic. In particular, dietary and micronutrinet supplementation has been a mainstay of the mitigation of arsenic toxicity. However, there has not been evidence base to justify if supplementation with Folate or selenium can alter the course of arsenic toxicity. Before expensive arsenic mitigation programs are undertaken to address the issue of arsenic toxicity, these issues need to be discussed. In elucidating the associations between diet, micronutrients and arsenic-caused skin lesions, and subsequently associations between methylation and arsenic-caused skin lesions and associations between diet, micronutrients and arsenic methylation, this dissertation aims to provide an evidence base from the perspective of environmental epidemiology. In summary, weaving evidence from environmental epidemiological studies, and translating the research in the language of health services research, this dissertation aims to create an interface between environmental epidemiology and health services research.

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medical care in a large variety of settings explained on an average (Median R square: 0.20, range: 0.01 - 0.85) 20 pct variation in the healthcare services utilization pattern. The most frequent variables for environmental factors included in these studies were rural or urban locations of patients. They criticized that while half of these studies were based on secondary data, the databases lacked inclusion of environmental variables beyond location data, and suggested needs for merging databases and conducting linked analysis.

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Table 1. Historical development of arsenic toxicity in West Bengal, and Bangladesh, India

Year or Range of years	Event	Impact
1960s	Increased prevalence of diarrheal diseases, decision to install tubewells at depths of 20-60 m	Arsenic in drinking water was not measured at that time
1982	Diagnosis of arsenicosis patients by Prof Kshitish Chandra Saha at School of Tropical Medicine, 16 patients in a cluster of villages in South 24 Parganas district of West Bengal	Incident cases of Arsenicosis, emergent problem
1986	West Bengal Government forms a committee to investigate the arsenicosis problem	Investigations into the topic of arsenicosis in West Bengal
1995	First cases of arsenic toxicity determined in Bangladesh	
1995-1996	Cross sectional survey of 7683 individuals initiated in West Bengal	
2001	United States EPA and the WHO revised maximum contaminant level of arsenic from 50 microgram/L to 10 micrograms/L	India and Bangladesh still acknowledged the 50 micrograms/L maximum contaminant level margins

Table 2. Distribution of Arsenic contaminated wells (estimate of 2004). Legend: ug/L indicates micrograms per liter of drinking water, sqkm = square kilometers

Table 3. Distribution of arsenic toxicities in West Bengal and Bangladesh (Ref: Chowdhury et al)

Parameter	**Bangladesh**	**West Bengal, India**
Total Area	148393	89192.4
Total Population(million)	120	68
Number of districts	64	18
Number of districts with As > 50	42	9

Area of the affected districts	92106	38865
Population of affected districts	79.9	42.7
Districts that have people with arsenical skin lesions	25	7

Table 4. Prevalence estimates for Arsenic caused skin lesions from studies conducted in India (West Bengal) and Bangladesh

Author,year	**population**	**size**	**skin lesions**	**prevalence**	**notes**
Rahman, 2006	Bangladesh	166934	504	3.02	cross-sectional survey of all eligible persons in a particular geographical area
McDonald, 2006	Bangladesh	13705	176	12.84	random sampling among women in 53 villages
Guha Mazumder, 1998	West Bengal, India	7683	361	46.9	Combined result for hyperpigmentation
Ahsan, 2006	Bangladesh	14828	714	48.15	Part of the health effects of arsenic longitudinal study
Rahman, 2003	West Bengal,India	33000	2274	68.91	cross-sectional survey with random selection
Chowdhury, 2000	West Bengal, India	29035	4420	152.23	cross sectional survey with voluntary recruitment of people with skin lesions
Mukherjee, 2005	West Bengal, India	25274	4813	190.43	cross-sectional survey based on volunteer response
Chowdhury, 2000	Bangladesh	11180	2736	244.72	cross sectional survey with voluntary recruitment of people with skin lesions
Tondel, 1999	Bangladesh	1481	430	290.34	cross sectional survey with nonsystematic sampling of wells and individuals above 30 years of age

Background

Methods

Results

Table 2: Demographic variables, socioeconomic status, and outcome variables

Table 3: Dietary variables and micronutrients mean (sd)

Table 4: Average nutritional factors between cases and controls

Table 5: Adjusted Ors for nutritional factors between cases and controls

Discussion

Association between dietary intakes, serum levels of micronutrients and arsenic-caused skin lesions: results from a case control study in West Bengal, India

Abstract

Background. -- Skin lesions are manifestations of arsenic toxicity. Earlier studies report that people with low intakes of specific dietary elements, or those with low serum levels of anti-oxidants are susceptible to arsenic-caused skin lesions. This is the first study to report combined effects of diet and micronutrients on susceptibility to arsenic-caused skin lesions.

Methods. -- We conducted a case-control study in an arsenic-exposed population in West Bengal, India. We selected age- and gender-matched cases and controls from a cross-sectional survey in West Bengal from individuals exposed to inorganic arsenic in their groundwater (upto 500 ug/L). We calculated their dietary intakes, and measured micronutrients from their blood samples. We regressed case-control status on quintiles of dietary intakes and serum micronutrients. In multivariate models, we adjusted for the effects of demographic, socioeconomic, arsenic-exposure, other dietary variables, and serum micronutrients as covariates.

Results. -- Cases and controls had similar housing ($p = 0.37$) and educational status ($p = 0.27$). Cases had lower levels of urinary inorganic arsenic excretion ($p = 0.01$), and lower dietary intakes of Vitamin A, Iron, Fiber, Animal Fat, Calcium, and lower serum Beta-cryptoxanthine. Compared with individuals in the highest quintiles, those in the lowest were about 3.5 times susceptible to skin lesions for dietary Vitamin A (OR=3.43;95% CI:1.19-9.90), 2.7 times for dietary Iron (OR=2.68;95% CI:1.34-5.35), 2.5 times for dietary fiber (OR=2.45; 95% CI:1.25- 4.81), and about twice for Animal Fat (OR = 2.1, 95% CI: 1.11 - 3.97), and beta- cryptoxanthine (OR = 2.07, 95% CI = 1.05 - 4.05). In multivariate models, low dietary intakes of Vitamin A, and low levels of serum beta cryptoxanthine were associated with increased susceptibility to skin lesions.

Discussion. -- Low Vitamin A intake and low levels of serum antioxidants are consistently associated with arsenic-caused skin lesions.

Conclusion. -- This study provides limited evidence that low dietary intakes of Vitamin A and low serum micronutrients may increase susceptibility to arsenic-caused skin lesions in exposed

population.

Background

More than 80 million people in the West Bengal state of India and its adjoining areas in Bangladesh in teh Ganges river delta area are exposed to inorganic arsenic in the groundwater exceeding 50 microgram/L (?ref). Skin lesions are protean manifestations in response to long term exposure to such high concentration of inorganic arsenic. These include alternate dark and light skin pigmentation on the trunk (melanosis) thickened dark skin pigmentation (keratosis), and nodular deformities (Guha Mazumder). Presence of skin pigmentation with evidence of exposure to high concentrations of inorganic arsenic (exceeding 50 ug/L) is hallmark of arsenic toxicity (??). Skin manifestations are initial manifestation of arsenic toxicity and in addition to skin pigmentation, prolonged arsenic exposure also results in skin cancer (Bowen's disease), and cancers of urinary bladder, lung, and liver (refs??). Arsenic exposure is also associated with non-malignant diseases, including diabetes, hypertension, chronic obstructive pulmonary diseases, and bronchiectasis, among others (refs??).

Diet and micronutrients play important roles in susceptibility to arsenic-caused skin lesions. In the 1960s, when studies from Southwestern Taiwan suggested that high exposure to inorganic arsenic was associated with increased risk of skin cancers, it was argued that susceptibility to arsenic toxicity among the Taiwanese population could be attributable to the dietary patterns of the Taiwanese that was rich in Tapioca and carbohydrates and low in protein and therefore made them more susceptible to arsenic-caused skin cancers, while relatively higher dietary protein intake of the US population might result in lower susceptibility for the US or a diet-for-diet comparable population. On the other hand, cross sectional studies among the Atacameno people in Chile, who are exposed to high concentrations of inorganic arsenic through natural water, but also have good nutritional status through generations, suggest that despite generations of high dietary protein intake and good nutrtional status, prevalence of arsenic caused skin lesions in this population was

comparable to those found in other geographical areas in the world which had comparable arsenic exposure (Smith). Nevertheless, results from population based cross-sectional surveys and case control studies from Southwestern Taiwan, Bangladesh and India continue to provide evidence that dietary deficiencies in protein, xxx, aaa, or low serum levles of beta carotene, methionine and xxx result in higher susceptibility to arsenic-caused skin lesions in these populations (refs??).

The purpose of this paper is to present results of our case control study on the combined effects of diet and micronutrients on arsenic caused skin lesions. This is the first study that considered the effects of dietary variables, together with serum micronutrients.

Methods

We conducted a population based case control study nested within a larger cross-sectional survey. The ascertainment of cases and controls, and the methods of assessment of dietary variables, and serum micronutrients are described below.

Study participants

We conducted a cross-sectional investigation of 180 cases who had skin lesions and 192 persons who did not have skin lesions, in the North 24 Parganas district, of the state of West Bengal in India, between 1998 and 2000.

Participants had been selected for a previously completed case-control study of arsenic-caused skin lesions [11]. The study base for selection of cases and controls involved 7683 individuals who participated in a 1995-1996 population based cross-sectional survey of the South 24 Parganas, a rural district located south of Calcutta. The survey identified 415 individuals with signs of arsenic-induced skin lesions [10]. Water samples were collected from the primary current drinking-water source of each participant. Because of our interest in examining effects at low doses, we based the case-control study on survey participants whose primary drinking water sources contained less than 500 micrograms/liter of inorganic arsenic (N = 4815, 2160 females and 2025 males). The study involved 180 cases and 192 controls. Cases were individuals who had a positive skin lesion classification, with either hyperpigmentation (mottled dark brown pigmentation bilaterally distributed on the trunk) or keratoses (diffuse thickening of palms or soles, with or without nodules) at the time of survey. Controls were randomly selected from the study base, and matched to the cases by gender and age within 4 years. The study protocol was approved by the Institutional Review Boards of the Institute of Post Graduate Medical Education and Research, Calcutta and the University of California, Berkeley. Informed consent was obtained from all participants.

The current investigation we report here uses cross-sectional data from the cases and controls including dietary assessment, measurement of blood micronutrients, and measurement of methylated arsenic species in urine samples. The controls were selected to be representative of the source population as described above. Cases with skin lesions are included in the present analysis to increase the numbers of participants. As explained below, we checked for distortion of associations between nutritional factors and arsenic methylation patterns due to the inclusion of both cases and controls.

Assessment of dietary intake and blood micronutrients

For each participant, we meaured weight and height and calculated the body mass index (BMI). We ascertained food intake for each participant with a detailed questionnaire based primarily on 24-hour recall. We have previously presented the methods used for dietary assessment [12]. In brief, the senior woman, who in this population directs preparing food for the family, was interviewed. The senior woman, was often the mother in the family or the eldest daughter-in-law, was questioned about each meal from the previous day's lunch through to the breakfast on the day of the interview. The volume of each cooked food was assessed by questioning the senior woman using standard cups and plates. Standard sized spoons were used to assess the intake of sugar and oil. We asked about weekly consumptions of meat, fish, eggs, milk, and fruit because these items were not consumed on a daily basis. The 1-week intake of these food items was then divided by 7 to compute the average intake per day. We calculated total 24 hour intake of each nutrient using a spreadsheet program based on food composition tables.

Field physicians interviewed participants using a structured questionnaire, conducted a general examination, and obtained blood samples from each participant when they were visited in their homes. We have previously presented detailed information concerning storage and analysis of blood samples [6]. In brief, nonfasting blood samples were collected and stored in an ice chest in the field.

Aliquots were prepared within 24 hours, frozen at -20 deg C in India, later transported to the United States on dry ice where they were stored at -70 deg C until laboratory analysis. Pacific Biometrics (Seattle, WA) conducted most serum and plasma analyses for the micronutrients and biochemical indicators, or in some instances arranged for them to be done at a different laboratory. Plasma measurements included Homocysteine, glutathione, cysteine, methionine, vitamin B6, retinol (Vitamin A), alpha-tocopherol (Vitamin E), alpha-carotene, beta-carotene, lycopene, lutein-zeaxanthin, and beta-cryptodextrin.

Serum measurements included glucose, cholesterol, vitamin B12, folate, transthyretin, and selenium.

Ascertainment of cases and controls

Ascertainment of cases and controls. – The survey team members questioned each participant about sources of drinking water, current diet, water intake, symptoms, height and weight. Each participant received a general medical examination, and survey team physicians carefully inspected for arsenic skin lesions.

We used the following criteria for classifying keratoses and hyperpigmentation as arsenic-caused. Keratoses had to involve bilateral diffuse thickening of palms and/or soles with or without nodules of various shapes and sizes. Hyperpigmentation were areas of mottled dark brown pigmentation bilaterally distributed on the trunk. Hyperpigmentation was frequently also present on the limbs, and sometimes alongside spots of depigmentation, but these characteristics were not regarded as essential for diagnosis. One of two field physicians in the survey team examined all patients in a well-lit area outdoors, under natural light.These physicians had about 10 years of experience each in diagnosing arsenic-caused skin lesions in West Bengal, including examining patients regularly in the Arsenic Clinic in the hospital linked with the Postgraduate Medical Institute in Kolkata. The physicians documented visible or palpable dermal lesions noting the location,

appearance, and whether the patterns were characteristic of arsenic-induced skin toxicity. Part-way through the project, interviewers photographed the most highly affected skin lesions. The Four project physicians later reviewed the slides. After joint review and discussions, the physicians classified the skin lesions (by consensus) as "definite", "probable", "possible", or "not related" to arsenic. Dermal changes "definite", or "probable" induced by arsenic were classified as current skin lesions. Participants, for whom slides were not available, were classified as having skin lesions if the physician interviewer recorded on the questionnaire that the dermal changes were of a type related to arsenic (Haque, 2003).

Field workers knew whether a village where they were working was located in the high or low exposure area. However, they had no information on the tubewell arsenic concentration at the time of working in the area. Physicians examined the participants only after they were first interviewed by the field workers. While the water samples were obtained on the same day as the interview and medical examinations, but the results of the analyses for arsenic were not known until months later. Thus the physical examinations for skin lesions were conducted blind as to water arsenic concentrations which showed a wide variability, with contaminated wells scattered irregularly throughout the study region.

We selected controls from survey participants who did not have skin keratoses or hyperpigmentation when seen during the 1995–1996 survey, and whose main tube well-water source, like the cases contained < 500 micrograms/liter of arsenic. For each case, we selected one control matched on age (plus or minus 5 years) and sex randomly from all eligible non-cases. We selected replacement controls for controls who had died, could not be located or did not wish to participate.

Statistical data analysis

This was an exploratory data analysis where the aim was to identify the set of dietary and serum micronutrients that were associated with skin lesion status. For every dietary variable, we adjusted

for the calorie intakes of the dietary variables by dividing them with their calorie intake for each individual (Willet). For dietary variables and serum micronutrients, all non-detectable values were considered as missing values and were removed from the analysis. We also converted earch dietary and micronutrient variable into its corresponding quintile form based on the values for controls (ie converted a continous variable into a categorical variable with five levels based on the lowest value, the 20th, 40th, 60th, 80th quintiles, and maximum values for those of the control population as break points to denote the cut-off values for levels). This was done for three reasons. First, doing so would make odds ratios comparable between the variables. Second, if outliers were included in the analyses, they would be incorporated within the lowest or the hightest quintiles and would not need to be separately treated or removed from the analysis, thus maintaining optimum power. Third, adjust for the effects of potential collinearity among individual variables when they were included in multivariate models.

We compared levels of educational attainment, and types of housing between cases and controls using chisquare tests of trend for contingency tables. For continuous variables, we compared median values of dietary variables, serum micronutrients, and indicators of methylation (urinary percentages of inorganic arsenic, monomethyl arsonous acid and dimethyl arsinic acids) between cases and controls. To test whether the differences in the median for these variables between cases and controls could be due to chance, we used Wilcoxon nonparametric tests of comparison and its associated p-value at 0.05 cut-off.

We used simple unconditional logistic regression with age and gender as co-variates for identifying the initial set of dietary variables and serum micronutrients that would be included in the subsequent mulivariate logistic regression models. Here, we regressed the outcome variable -- the case-control status -- on each dietary and serum micronutrient variable in turn, and compared the exponentialized standardized beta of the generalized linear model (the Odds Ratio) along with the

95% Confidence Interval bands (the lower and the upper limits of the 95% confidence interval of each dietary variable and serum micronutrient). After the regression was run through the entire list of dietary and micronutrient variables, they were rank ordered in descending absolute values of their beta coefficients.

We selected further variables for multivariate models from the list based on their beta coefficient (and therefore Odds Ratio estimates) and whose 95% confidence intervals did not include unity. The variable that had the highest Odds Ratio was then combined with the variable that had the next highest Odds Ratio in the descending order and was put into the multivariate logistic regression model with the following co-variates: age, gender, housing status, educational attainment, and total urinary arsenic in urine.

Results

For both cases and controls, about 60% individuals were less than 40 years old, and more than 60% were men. Cases and controls were comparable in their housing accommodation types and educational attainments. About 51% lived in cobs or houses made with clay and straw; 30.7% cases and 27.2% controls had no formal education (Table 1). Cases had lower InAs% and higher DMA% in their urine, indicating that their overall methylation capacities were higher than controls (Table 2).

For dietary intakes of Vitamin A, Iron, Fibre, Animal Fat and Beta-cryptoxanthine, compared to individuals who were in the highest quintiles of either dietary intakes or serum concentrations (as in case of Beta-cryptoxanthine), individuals who were in the lowest quintiles were at higher risk of skin lesions. In the unadjusted models, compared to those in the highest quintiles of dietary intakes of vitamin A, those who were in the lowest quintiles of Vitamin A dietary intakes were about 23% more likely to suffer from arsenic-caused skin lesions (Odds Ratio: 1.23, 95% Confidence interval: 1.19 - 9.90). Compared to those individuals who were in the highest quintiles of dietary iron intakes, those who were in the lowest quintiles were about 2.7 times likely (OR = 2.68; 95% CI: 1.34 - 5.35), compared to those in the highest quintiles of dietary fibre intakes, those who were in the lowest quintiles were about 2.5 times as likely (OR = 2.45; 95% CI: 1.25 - 4.81), people in the lowest quintiles for dietary Animal Fat were 2.1 times (OR = 2.10; 95%CI: 1.11 - 3.97), and those who were in the lowest quintiles of dietary calcium intakes were 1.9 times at risk of developing skin lesions (OR = 1.91; 95% CI: 1.03 - 3.52).

When these variables were added sequentially to the multivariate logistic regression models, we found that even after adjustment of all other variables and other co-variates, dietary Vitamin A and serum beta cryptoxanthine were the only variables that maintained their association with the case control status.

Discussion

We found that when the dietary variables and serum micronutrients were considered together in a multivariate model containing demographic variables, socioeconomic status, arsenic exposure variables, and other dietary and micornutrients, dietary intakes of vitamin A and serum beta cryptoxanthine were associated with increased risk of arsenic-caused skin lesions among arsenic-exposed population. Compared to those who were in the highest quintiles of dietary Vitamin A intakes, those who were in the lowest quintiles were 3.43 times at risk of skin lesions (OR = 343, 95% CI: 1.19 -9.90), and compared to those who were in the highest quintiles of serum Betacryptoxanthine levels, those who were in the lowest levels were about twice at risk of skin lesions (OR = 2.07, 95% CI: 1.05 - 4.05). These associations were not influenced even after adjustment for the effects of housing status, educational attainment, indicators of arsenic exposure and effects of all other dietary variables and serum micronutrients. Additionally, when the quintiles of dietary intakes of Vitamin A and beta cryptoxantine were plotted against the odds ratios of skin lesions, they showed a decreasing slope of the risk with increase in the quintiles of dietary Vitamin A and serum betacryptoxanthine.

This study derived its strength from its large sample size, nesting of cases and controls within a large cross-sectional survey that was aimed to minimize possible measurement biases, and use of multivariate logistic regression incorporating quintiles of dietary and serum micronutrients together in the same model with other co-variates. The study was based on on 180 cases and 192 controls, derived from a large cross sectional survey of about 7683 individuals, who were examined in a house to house survey. The dietary status of each individual enrolled in the study was ascertained from the eldest female member of the family, or the person who prepared the food for the family. The process of determination of serum levels of micronutrients was separate from the process of field data collection. Physicians, who determined the skin lesion status in the clinics following data collection from the fields, had no knowledge of the dietary status of the participants. Finally, since

this was a population with low literacy levels, respondents had no knowledge about the dietary constituents of their food item.

Other studies on only dietary or micronutrient variables have also found that low dietary intakes of vitamin A and low serum levels of antioxidants (betacryptoxanthine) as risk factors.

Conclusion

References

Table 1. Comparisons of cases and controls with respect to their age distribution, gender, housing and educatioal attainment as measures of demographic and socioeconomic status. The pvalues in this table indicate p value for the one way test of trend in the percentages for cases and controls

Variable	Category	Controls	%	Cases	%	pvalue
Age in years						Matched
	less than 20	34	16.04	27	14.06	
	20-39	82	38.68	83	43.23	
	40-59	70	33.02	60	31.25	
	60 and above	26	12.26	22	11.46	
Gender						Matched
	female	76	35.68	73	38.02	
	male	137	64.32	119	61.98	
House type						0.37
	kacha	110	51.64	99	51.56	
	pucca	28	13.15	30	15.63	
	semipucca	75	35.21	63	32.81	
Educational Status						0.27
	no formal	58	27.23	59	30.73	
	high school and above	11	5.16	9	4.69	
	primary	103	48.36	97	50.52	
	secondary	41	19.25	27	14.06	

Table 2. Comparison between percentiles of percent fractions of excreted inorganic arsenic, MMA, and DMA in urine between cases and controls. P values are based on the one way test of trend

Variable	Quintile	Cases	%	Controls	%	pvalue
InAs%						0.01
	Lowest	49	25.52	31	14.83	
	Q2	43	22.4	37	17.7	
	Q3	32	16.67	48	22.97	
	Q4	33	17.19	47	22.49	
	Highest	35	18.23	46	22.01	
MMA%						0.3
	Lowest	33	17.19	47	22.49	
	2	43	22.4	37	17.7	
	3	37	19.27	43	20.57	
	4	40	20.83	40	19.14	

	Highest	39	20.31	42	20.1	
DMA%						0.06
	Lowest	36	18.75	44	21.05	
	Q2	31	16.15	49	23.45	
	Q3	40	20.83	40	19.14	
	Q4	37	19.27	43	20.57	
	Highest	48	25	33	15.79	

Table 3. List of dietary and serum micronutrient variables and their differences in median, 10th and 90th percentiles between cases and controls. The p-values are based on nonparametric Wilcoxon rank sum tests.

	Cases			Controls			
Variables	Median	10th percentile%	90th percentile%	Median	10th percentile%	90th percentile%	p-value
Serum Glucose	81.5	53.8	129.1	81	55	113	0.09
Serum Cholesterol	153.5	110.9	198	149	114	207.4	0.49
Serum Transthyretin	235	162.7	307	240	173	306	0.19
Serum Vitamin B12	376	218.7	712	383	216.4	674.4	0.43
Folate	2.85	1.35	5.6	2.6	1.5	5.6	0.1
Selen	1.15	0.71	1.73	1.15	0.66	1.75	0.25
Cysteine	214	175.6	260	212	166	261	0.3
GlutGSSG	2.76	1.29	4.98	2.32	1.16	5.09	0.11
GlutGSH	5.52	2.57	9.95	4.64	2.32	10.17	0.11
Homocyst	12.7	8.18	21.16	13	8.63	23.53	0.22
Retinol	32.8	19.02	47.86	34	21.5	48.44	0.13
Atoc	597	439.6	870.6	594.5	408.1	910.4	0.42
LutZea	61	34	99.6	61.5	32.6	104	0.4
Bcrypt	3.86	1.76	13.7	4	1.5	11.12	0.39
Lycopene	2	0.55	6.8	1.8	0.45	7.87	0.44
BetaCar	55.9	18.88	220.14	50.7	21.06	132.05	0.19
Vit.B6	34.3	20	51.5	34.95	21.8	58.22	0.05
Methion	19.15	13.11	26.33	18.8	12.5	26.8	0.31
Proteinan	3.32	0.81	8.66	4.1	1.32	9.41	0.01
Protnveg	19.6	16.77	23.55	19.34	16.59	25.03	0.27

Fatanimal	0.93	0.17	5.11	1.27	0.18	4.53	0.14
Fatveg	8.53	3.64	17.02	8.35	4.39	17.5	0.38
carbohydr	203.22	175.64	218.23	201.91	174.09	214.6	0.12
fibre	1.93	1.09	3.49	2.1	1.1	4.59	0.04
calcium	175.36	71.28	402.32	210.93	99.29	405.2	0
phosph	467.05	406.12	641.46	481.22	413.13	657.46	0.07
iron	5.59	3.89	8.69	5.9	3.75	9.98	0.12
zinc	4.08	3.42	4.69	4.01	3.37	4.86	0.13
carotene	271.56	47.12	4294.55	344.35	52.23	6070.76	0.1
VitA	12.36	0	72.18	14.55	0	64.43	0.4
thyamine	0.63	0.56	0.83	0.63	0.55	0.87	0.18
riboflavin	0.25	0.16	0.48	0.26	0.16	0.45	0.14
niacin	9.92	8.28	10.82	10.01	8.11	10.81	0.48
VitB6	0.58	0.43	0.68	0.56	0.4	0.71	0.13
Folicfree	26.96	21.68	41.42	28.05	20.48	42.13	0.34
Folictotal	61.25	37.01	119.83	65.53	38.15	129.77	0.06
VitC	31.7	11.85	100.99	37.28	13.75	134.57	0.03
Totalnitr	2.68	1.85	3.39	2.62	1.73	3.25	0.13
arginine	1544.92	924.04	1933.86	1491.73	865.73	1846.83	0.05
histidine	478.82	312.2	613.83	468.22	282.46	596.85	0.09
lysine	723.58	507.15	1061.36	698.9	464.1	1037.43	0.16
tryptophan	193.71	137.24	250.37	189.36	123.93	247.7	0.11
phenylana	899.75	597.99	1123.86	880.68	533.63	1094.99	0.06
tyrosine	731.35	484.37	912.74	711.47	438.99	887.08	0.05
methionin	497.21	332.92	617.22	482.83	286.19	606.33	0.13
cystine	247.55	165.38	306.24	246.42	149	305.65	0.07
threonine	745.93	507.35	935.7	726.88	442.53	917.24	0.09
leucine	1429.34	962.99	1819.52	1400.09	887.57	1765.49	0.07
isoleucine	821.87	565.35	1056.1	809	523.15	1047.68	0.12
valine	1147.87	761.75	1424.45	1119.62	668.79	1398.3	0.07

Table 4. Results of single variable logistic regression where case control status was regressed on the standardized dietary variables and serum micronutrients after controlling for the effects of age in years, and gender. Standardized beta coefficients and associated pvalues for the regression models are shown here, along with mean and standard deviations of the corresponding variables for the controls.

	Mean	SD	Beta	Pvalue
Glucose	82.056	26.402	0.222	0.049

Chol	155.777	36.065	-0.076	0.489
Trans	239.563	49.258	-0.093	0.382
Vit.B12	439.636	382.256	-0.047	0.663
Folate	3.346	3.084	0.020	0.853
Selen	1.163	0.430	0.044	0.673
Cysteine	214.170	37.988	0.019	0.868
GlutGSSG	3.240	3.332	-0.062	0.561
GlutGSH	6.480	6.664	-0.062	0.561
Homocyst	15.047	8.398	-0.070	0.520
Retinol	34.766	10.640	-0.134	0.201
Atoc	634.351	216.530	-0.010	0.928
LutZea	66.928	31.235	-0.042	0.692
Bcrypt	5.534	4.716	0.064	0.552
Lycopene	3.612	5.805	-0.132	0.247
BetaCar	69.072	61.923	0.368	0.005
Vit.B6	44.597	60.724	-0.436	0.090
Methion	19.321	5.824	0.042	0.695
Proteinan	4.817	3.394	-0.168	0.109
Protnveg	19.991	3.598	-0.005	0.962
Fatanimal	1.904	1.999	-0.023	0.821
Fatveg	10.095	6.296	-0.083	0.409
carbohydr	197.712	16.679	0.115	0.263
fibre	2.572	1.638	-0.281	0.010
calcium	236.305	140.463	-0.199	0.061
phosph	512.153	107.457	-0.146	0.160
iron	6.521	2.768	-0.206	0.053
zinc	4.062	0.622	0.078	0.437
carotene	1750.403	3277.582	-0.125	0.225
VitA	25.239	32.034	-0.019	0.850
thyamine	0.666	0.134	-0.023	0.820
riboflavin	0.289	0.122	-0.046	0.653
niacin	9.716	1.111	0.032	0.748
VitB6	0.556	0.122	0.086	0.387
Folicfree	30.380	10.270	0.012	0.901
Folictotal	77.433	42.208	-0.174	0.093
VitC	57.208	58.809	-0.251	0.024
Totalnitr	2.573	0.658	0.122	0.233
arginine	1437.403	384.645	0.166	0.107

histidine	458.452	122.944	0.141	0.165
lysine	737.164	253.095	0.074	0.462
tryptophan	190.858	53.430	0.116	0.250
phenylana	851.369	217.381	0.159	0.120
tyrosine	691.279	177.937	0.167	0.103
methionin	468.611	123.783	0.139	0.177
cystine	236.579	67.271	0.137	0.182
threonine	708.693	182.854	0.140	0.171
leucine	1373.843	358.960	0.151	0.140
isoleucine	796.739	209.481	0.123	0.225
valine	1081.563	272.586	0.158	0.122

Table 5. Correlation matrix of the seven highest associated (in terms of the standardized betas in logistic regression model) dietary variables and serum micronutrients

	Vit.B6	BetaCar	fibre	VitC	iron	calcium	Folictotal
Vit.B6	1	0.1	-0.01	-0.04	0.03	0.07	-0.01
BetaCar	0.1	1	-0.01	-0.03	0.05	0.35	0.08
fibre	-0.01	-0.01	1	0.73	0.32	0.31	0.27
VitC	-0.04	-0.03	0.73	1	0.24	0.23	0.3
iron	0.03	0.05	0.32	0.24	1	0.44	0.59
calcium	0.07	0.35	0.31	0.23	0.44	1	0.51
Folictotal	-0.01	0.08	0.27	0.3	0.59	0.51	1

Table 5. Results of multivariate logistic regression where case control status was regressed on the standardized selected dietary variables and serum micronutrients after controlling for the effects of age, gender, housing, educational attainment, urinary percentage of INAS and MMA.

	S. Vitamin B6	S. Betacarotene	D.Fiber	D.Vitamin C	D. Iron	D. Calcium	D. Folate
Univariate	-0.436 (0.090)	0.368 (0.004)	-0.281 (0.010)	-0.251 (0.024)	-0.201 (0.051)	-0.199 (0.060)	-0.174 (0.093)
M1	0.065 (0.546)	0.369 (0.004)					
M2		0.366 (0.005)	-0.310 (0.008)				
M3		0.361 (0.006)	-0.234 (0.145)	-0.111 (0.494)			

M4		0.356 (0.007)		-0.274 (0.021)			
M5		0.378 (0.004)	-0.255 (0.039)		-0.157 (0.181)		
M6		0.372 (0.005)		-0.221 (0.069)	-0.187 (0.116)		
M7		0.473 (0.001)	-0.232 (0.059)			-0.288 (0.037)	
M8		0.476 (0.001)		-0.197 (0.101)		-0.304 (0.025)	
M9		0.465 (0.001)	-0.221 (0.077)			-0.239 (0.126)	-0.086 (0.515)
M10		0.390 (0.003)	-0.256 (0.034)				-0.183 (0.120)

The following is an alternative analysis

Table 3. Results of single variable models. The first column gives the beta coefficient between the highest quintile and the lowest quintile. Here, the highest quintile has been the baseline. Thus the first column figure represents compared to those in the highest quintiles of dietary intakes or serum micronutrient levels, those who were in the lowest 20th percentiles, how likely were they to report with skin lesions. The second column represents the odds ratio and the associated 95% confidence intervals

	X1	X2	X3	X4
Glucose	0.298	1.348	0.719	2.527
Chol	0.238	1.269	0.679	2.371
Trans	-0.033	0.968	0.523	1.791
Vit.B12	0.364	1.439	0.769	2.693
Folate	0.524	1.689	0.875	3.260
Selen	0.500	1.648	0.862	3.153
Cysteine	0.197	1.218	0.616	2.408
GlutGSSG	-0.087	0.917	0.458	1.834
GlutGSH	-0.087	0.917	0.458	1.834
Homocyst	-0.939	0.391	0.205	0.746
Retinol	-0.138	0.871	0.452	1.678

Atoc	-0.067	0.936	0.471	1.857
LutZea	0.091	1.095	0.561	2.136
Bcrypt	0.725	2.065	1.054	4.047
Lycopene	-0.388	0.678	0.341	1.349
BetaCar	-0.028	0.972	0.533	1.775
Vit.B6	-0.424	0.654	0.325	1.316
Methion	0.082	1.085	0.560	2.104
Proteinan	0.470	1.599	0.849	3.012
Protnveg	-0.226	0.798	0.414	1.537
Fatanimal	0.740	2.096	1.107	3.967
Fatveg	0.536	1.709	0.932	3.132
carbohydr	-0.404	0.668	0.368	1.211
fibre	0.896	2.450	1.250	4.805
calcium	0.645	1.905	1.031	3.519
phosph	0.427	1.532	0.821	2.859
iron	0.984	2.676	1.338	5.350
zinc	-0.135	0.873	0.453	1.685
carotene	0.300	1.350	0.716	2.545
VitA	1.234	3.434	1.191	9.897
thyamine	-0.188	0.829	0.440	1.562
riboflavin	0.328	1.388	0.755	2.552
niacin	-0.589	0.555	0.296	1.039
VitB6	-0.546	0.579	0.306	1.097
Folicfree	-0.159	0.853	0.452	1.610

Folictotal	0.530	1.699	0.891	3.237
VitC	0.606	1.834	0.949	3.543
Totalnitr	-0.581	0.559	0.299	1.045
arginine	-1.181	0.307	0.160	0.588
histidine	-0.853	0.426	0.227	0.799
lysine	-0.349	0.705	0.374	1.330
tryptophan	-0.784	0.457	0.241	0.864
phenylana	-0.717	0.488	0.262	0.908
tyrosine	-0.688	0.502	0.270	0.935
methionin	-1.210	0.298	0.153	0.581
cystine	-1.149	0.317	0.169	0.595
threonine	-0.894	0.409	0.219	0.765
leucine	-0.743	0.475	0.254	0.890
isoleucine	-0.747	0.474	0.253	0.887
valine	-0.840	0.432	0.229	0.812

CHAPTER 4: NUTRITIONAL FACTORS AND METHYLATION

Abstract

Exposure ot inorgnic arsenic through groundwater supply results in considerable illnesses including debilitating skin lesions, cancers, and other non-cancerous diseases. It is believed that high monomethyl arsonous acid (MMA) is associated with altered DNA methylaiton and leads to cancerous and other adverse effects. Although methylaiton is influenced by micronutrients and it is understood that nutritional factors may impact arsenic toxicity, the association between different nutritional factors taken together and arsenic methylation patterns is not clear. The purpose of this study was to report the impact of nutritional factors on methylationof arsenic.

A case control study of 192 individuals with arsenic caused skin lesions matched with 213 controls was conducted within a large cross sectional survey in the arsenic-endemic regions of West Bengal state of India. A total of 23 nutritional factors were assessed on the tertiles of the three indicators of arsenic methylation (InAs%, MMA%, and DMA%). A two step examination of the association between nutritional factors and indicators of arsenic methylation was conducted. In the first step, each of hte three indicators were regressed on the nutritional factors individually; in step two, the nutritional factors that were important inthe step I were entered in a series of stepwise multiple linear regression models and were assessed for their impact on indicators of arsenic methylation.

Dietary animal fat, serum folate, serum selenium, and plasma retinol were found to be importantly associated with MMA%, while lycoepne was fount to be associated with InAs%.

These findings suggest that nutritional factors play important roles in the pathway linking methylation and health effects and it is possible to address arsenic methylatoin and possibly contain arsenic caused skin lesions by nutitional supplementation and other programmes. The role of dietary animal fat, arsenic methylaiton and arsenic caused health effects is reported for the first time

and the impact is unclear.

Introduction

Methods

Discussion

Table 1. Demographic, socioeconomic, total urinary arsenic and BMI. Total number N=405.

Characteristics	N	% of N	InAs %	MMA %	DMA %
Age in years					
<15	28	6.9	20.4	6.75	72.8
15-29	91	22.5	23.3	8.41	68.3
30-44	135	33.4	23.6	8.31	68.1
45-59	89	21.9	24.8	7.89	67.3
≥60	62	15.3	19.5	9.34	71.2
		p[a]	0.19	0.06	0.14
Gender					
Female	154	38.2	24.5	7.02	68.5
Male	251	61.8	22.2	8.88	68.9
		p[b]	0.05	0.01	0.2
Education					
Non-formal	117	28.9	22.5	8.24	69.3
Primary	200	49.5	23.5	8.23	68.3
Secondary	68	16.8	22.8	7.95	69.2
High School	20	4.9	22.4	8.33	69.3
		p[a]	0.47	0.48	0.46
House-type					
Kacha	205	50.6	22.6	8.33	69.1
Semi-pucca	138	34.1	24.1	7.85	68.1
Pucca	58	14.3	22.3	8.51	69.2
Missing data	4	0.9			
		p[a]	0.32	0.25	0.4
Skin Lesions					
Present	213	52.5	21.2	8.32	70.4

Absent	192	47.4	24.7	8.07	67.2
		p^b	0.02	0.56	0.03
BMI					
<16.9	122	30.1	23.5	7.96	68.5
16.9-19.3	121	29.8	21.3	8.73	70.1
>19.3	126	31.1	22.2	8.35	69.4
Missing	36	8.9			
		p^a	0.52	0.42	0.73
Total urinary arsenic (µg/L)					
<14.9	134	33.1	20.4	8.46	71.1
14.9-56.3	133	32.8	22.5	7.84	69.6
>56.3	138	34.1	21.5	8.51	69.9
		p^a	0.55	0.48	0.71

p^a p-value from test for trend.
p^b p-value from t-test.

Table 2. Average intake per day of dietary factors, average plasma and serum concentrations of micronutrients, and average urine creatinine concentrations with standard deviations (SD).

Dietary intake per day	Average	(SD)	Micronutrients	Average	(SD)
Diet survey			Plasma		
Calcium (mg/day)	484	(332)	Alphatocopherol (micro/dl)	636	(214)
Carbohydrate (g/day)	448	(161)	Betacarotene (microg/dl)	83.6	(97.3)
Carotene (microg/day)	3546	(7,161)	Betacryptoxanthin (microg/dl)	5.74	(5.61)
Energy (kJ/day)	9204	(3,091)	Homocysteine (micromol/l)	14.8	(8.1)
Fat, animal (g/day)	3.91	(4.24)	Lutein-Zeaxanthine (microg/dl)	66.5	(30.6)
Fat, vegetable (g/day)	21.8	(14.9)	Lycopene (microg/dl)	3.34	(4.92)
Fibre (g/day)	5.27	(3.47)	Methionine (micromol/l)	19.4	(5.72)
Folate (microg/day)	164	(102)	Retinol (micromol/dl)	34.1	(10.9)
Iron (mg/day)	13.9	(7.04)	Transthyretin (mg/l)	237	(53.1)
Niacin (mg/day)	22	(8.1)	Vitamin B6 (nmol/l)	40.8	(45.3)
Phosphorus (mg/day)	1130	(435)	Serum		
Protein, animal (g/day)	9.7	(7.14)	Cholesterol (mg/dl)	155	(35.4)
Protein, vegetable (g/day)	45	(17.1)	Cysteine (micromol/l)	215	(37.5)
Retinol (microg/day)	52.2	(66.1)	Folate (ng/ml)	3.38	(2.84)
Riboflavin (mg/day)	0.63	(0.33)	Selenium (micromol/l)	1.17	(0.41)
Thiamin (mg/day)	1.49	(0.56)	Total Glutathione (micromol/l)	6.31	(5.56)
Vitamin B6 (mg/day)	1.27	(0.52)	Vitamin B12 (pg/ml)	435	(315)
Vitamin C (mg/day)	111	(115)	Urine		
Zinc (mg/day)	9.17	(3.3)	Urine Creatinine (mg/l)	622	(531.8)

Table 3. Association between dietary variables, micronutrients, and indicators of methylation assessed by comparing the average arsenic metabolite percent between the lowest (T1) and highest (T3) tertiles of each factor with the p-value from the t-test of the mean difference.

Metabolite	Nutritional factor	Q1	Q3	Difference	P-value
InAs%	Urine creatinine	27.70	19.80	-7.81	0.001
	Plasma lycopene	26.33	20.47	-5.86	0.01
	Dietary riboflavin	20.92	25.17	4.25	0.04
	Serum selenium	19.63	23.74	4.11	0.03
	Serum folate	20.36	24.25	3.89	0.04
	Plasma vitamin B6	23.49	20.36	-3.13	0.14
	Dietary calcium	22.97	25.91	2.94	0.19
	Serum lutein-zeaxanthine	23.89	20.95	-2.94	0.14
MMA%	Dietary animal fat	7.000	9.330	2.330	0.001
	Serum retinol	7.324	9.232	1.908	0.001
	Serum homocysteine	7.315	9.179	1.864	0.002
	Dietary retinol	7.320	9.133	1.813	0.004
	Dietary animal protein	6.964	8.661	1.696	0.003
	Serum folate	9.633	7.955	-1.678	0.007
	Urine creatinine	7.210	8.720	1.510	0.004
	Serum selenium	9.204	7.741	-1.462	0.016
	Serum alphatocopheral	7.973	9.107	1.134	0.066
	Serum betacryptoxanthine	7.304	8.357	1.053	0.072
	Serum vitamin B6	7.729	8.718	0.989	0.106
	Serum methionine	7.821	8.761	0.940	0.109
	Dietary phosphorus	7.246	8.096	0.850	0.145
	Dietary carbohydrate	8.570	7.757	-0.814	0.147
DMA%	Urine creatinine	64.20	71.40	7.22	0.001
	Dietary riboflavin	70.54	65.94	-4.60	0.03
	Serum lycopene	65.35	69.62	4.27	0.07
	Serum lutein-zeaxanthine	65.94	69.90	3.96	0.06

Table 4. Results of multivariate analysis of nutritional factors measured in plasma (p), serum(s) diet(d) and in urine(u) with indicators of methylation in the model (covariates included in all models: age, gender, housing, education, skin lesion status, total urinary arsenic, and BMI). The beta coefficients correspond to that of the highest tertile (reference category: lowest or first tertile of the corresponding nutritional factor).

Model	Nutritional factor	beta coefficient	P-value	Model	Nutritional factor	beta coefficient	P-value
InAs Models				MMA Models			
InAs1	creatinine(u)	-4.56	0.08	MMA1	creatinine(u)	0.66	0.35
	lycopene(p)	-4.91	0.03		animal fat(d)	2.27	0.001
InAs2	creatinine(u)	-4.10	0.14	MMA2	creatinine(u)	0.84	0.26
	lycopene(p)	-6.23	0.02		animal fat(d)	2.01	0.003
	riboflavin(d)	5.89	0.02		retinol(p)	1.52	0.03
InAs3	creatinine(u)	-3.38	0.20	MMA3	creatinine(u)	0.95	0.24
	lycopene(p)	-5.25	0.04		animal fat(d)	1.88	0.01
	riboflavin(d)	4.61	0.07		retinol(p)	1.42	0.05
	selenium(s)	2.45	0.35		homocysteine(s)	0.73	0.35
				MMA4	creatinine(u)	0.87	0.28
DMA Models					animal fat(d)	2.18	0.003
DMA1	creatinine(u)	5.39	0.04		retinol(p)	1.34	0.07
	riboflavin(d)	-3.29	0.17		folate(s)	-0.96	0.21
DMA2	creatinine(u)	5.67	0.04	MMA5	creatinine(u)	0.56	0.46
	riboflavin(d)	-3.77	0.15		animal fat(d)	2.25	0.002
	lycopene(p)	3.91	0.13		retinol(p)	1.69	0.002
					selenium(s)	-1.56	0.03
				MMA6	creatinine(u)	0.52	0.53
					animal fat(d)	2.51	0.001
					retinol(p)	1.59	0.04
					folate(s)	-0.61	0.44
					selenium(s)	-1.83	0.02

Table 6: Demographic, socioeconomic, methylation and skin lesion status

Table 7: Association between nutritional factors and methylation

Table 8: Results of multivariate linear regression models between nutritional factors and methylation

Discussion

Draft Paper (this article is planned to be submitted to Environmental Health Perspectives. The references are not yet properly formatted according to EHP styles, but will be formatted once additional refs are added and the paper is finalized. Apologies for inconvenience. – Arindam Basu)

Relationship between diet, micronutrients, and patterns of arsenic methylation in West Bengal, India

Abstract

Background: Methylation may have an important role in arsenic toxicity based on evidence that the monomethylated trivalent metabolite (MMAIII) is more toxic than inorganic arsenic itself. It is therefore important to identify factors that might increase concentrations of MMA by reducing further methylation to dimethylated arsenic (DMA).

Objectives: To investigate the relationship of nutritional factors with methylation patterns of arsenic reflected in urine in what we believe is the first comprehensive joint assessment of dietary factors and blood micronutrients in a study including persons with and without arsenic skin lesions.

Methods: We conducted a a cross-sectional survey of an arsenic-exposed population in West Bengal including 192 persons with aresnic-caused skin lesions and 213 persons without. We assessed 30 dietary factors obtained from 24-hour recall, and 16 blood micronutrients. Bivariate and multivariate linear regression models were used to identify associations of indicators of arsenic methylation in urine with dietary factors and serum micronutrients, adjusting for age, gender, socioeconomic variables, body mass index, total urinary arsenic and skin lesion status.

Results: The strongest associations found involved MMA% which was positively related to the intake of animal fat (standardized beta = 0.249; p = 0.001), and negatively related to serum folate (standardized beta = -0.246; p = 0.001). MMA% was also positively associated with serum homocysteine concentrations (p=0.03), but this associaton disappeared when serum folate was adjusted for in the model.

Conclusions: This study provides further evidence that folate intake reduces concentrations of

MMA. The finding that high intake of animal fat increases MMA concentrations is new.

[Note to Allan: I deleted the reference to the opinion that it could be related to fish consumption because we did not find any association between fish related fat and any of the indicators of aresnic methylation. Indeed, the association between animal fat intake and arsenic methylation remains enigmatic]

Introduction

There is considerable in vitro evidence suggesting that monomethylated trivalent arsenic (MMAIII) is a highly toxic metabolite produced following ingestion of inorganic arsenic [20, 10]. Factors that influence arsenic methylation may therefore be important determinants of susceptibility to health effects resulting from the presence of inorganic arsenic in drinking water. In the state of West Bengal, India and adjoining Bangladesh, about 80 million individuals have been exposed to high concentrations of inorganic arsenic in tubewell water [3, 6, 11]. In view of widespread poor nutrition, dietary factors which might increase arsenic toxicity are an important topic for study in this population.

Inorganic arsenic in drinking water exists in trivalent, and pentavalent forms, As(III) and As(V). After ingestion, As(V) is reduced to As(III), followed by sequential reduction-methylation reaction steps resulting in formation of MMA(III), MMA(V), DMA(III) and DMA(V) [1, 12]. However, this process is incomplete, and considerable variability exists within populations in the proportions of inorganic arsenic and the methylated arsenicals excreted in urine. Methylation was once believed to be a process of detoxification of arsenic [7, 1], but current evidence following isolation and characterization of MMA(III) indicates that the first step of methylation which produces of MMA should really be thought of as an activation step, while the conversion of MMA to DMA in a second methylation step would reduce toxicity. In addition to the in vitro toxicity studies, recent human studies have found evidence of increased risks of skin and bladder cancer related for persons with higher proportions of MMA in their urine [4]. Human data concerning MMAIII itself are difficult to obtain because it is unstable and rapidly oxides to MMA(V), but it is reasonable to believe that persons with high concentrations of total MMA in urine could also have had high internal concentration of MMA(III) [2, 1]

Evidence is mounting that dietary constituents and blood micronutrients are related to the methylation of inorganic arsenic. We previously reported on the relationship between diet and

methylation in a study in the Western United States and found low protein intake associated with an increased proportion of arsenic as MMA in urine [18]. A study in Bangladesh involving plasma micronutrients found low folate and high homocysteine concentrations to be associated with higher proportions of MMA in urine [8], and a dietary study found that reduced intake of folate related nutrients were associated with higher proportions of MMA [17]. Furthermore, folic acid supplementation with concurrent exposure to arsenice in water resulted in a decrease in the proportion arsenic which was in the form of MMA [9]

We have previously reported on the relationship of dietary constituents [12] and blood micronutrients with arsenic-caused skin lesions in West Bengal [6]. Here, we report the findings concerning 46 dietary constituents and blood micronutrients with arsenic methylation patterns in urine in what we believe is the first comprehensive joint assessment of a large number of both dietary and micronutrient factors, including involving both persons with and without skin lesions.

Methods

Study participants

We conducted a cross-sectional investigation of 180 cases who had skin lesions and 192 persons who did not have skin lesions, in the North 24 Parganas district, of the state of West Bengal in India, between 1998 and 2000.

Participants had been selected for a previously completed case-control study of arsenic-caused skin lesions [11]. The study base for selection of cases and controls involved 7683 individuals who participated in a 1995-1996 population based cross-sectional survey of the South 24 Parganas, a rural district located south of Kolkata. The survey identified 415 individuals with signs of arsenic-induced skin lesions [10]. Water samples were collected from the primary current drinking-water source of each participant. Because of our interest in examining effects at low doses, we based the case-control study on survey participants whose primary drinking water sources contained less than 500 micrograms/liter of inorganic arsenic (N = 4815, 2160 females and 2025 males). The study involved 180 cases and 192 controls. Cases were individuals who had a positive skin lesion classification, with either hyperpigmentation (mottled dark brown pigmentation bilaterally distributed on the trunk) or keratoses (diffuse thickening of palms or soles, with or without nodules) at the time of survey. Controls were randomly selected from the study base, and matched to the cases by gender and age within 4 years. The study protocol was approved by the Institutional Review Boards of the Institute of Post Graduate Medical Education and Research, Kolkata and the University of California, Berkeley. Informed consent was obtained from all participants.

The current investigation we report here uses cross-sectional data from the cases and controls including dietary assessment, measurement of blood micronutrients, and measurement of methylated arsenic species in urine samples. The controls were selected to be representative of the source population as described above. Cases with skin lesions are included in the present analysis to

increase the numbers of participants. As explained below, we checked for possible distortion of associations between nutritional factors and arsenic methylation patterns due to the inclusion of both cases and controls.

Assessment of dietary intake and blood micronutrients

For each participant, we meaured weight and height and calculated body mass index (BMI). We ascertained food intake for each participant with a detailed questionnaire based primarily on 24-hour recall. We have previously presented the methods used for dietary assessment [12]. In brief, the senior woman, who in this population directs preparing food for the family, was interviewed. The senior woman, who was usually the mother in the family or the eldest daughter-in-law, was questioned about each meal from the previous day's lunch through to the breakfast on the day of the interview. The volume of each cooked food was assessed by questioning the senior woman using standard cups and plates. Standard sized spoons were used to assess the intake of sugar and oil. We asked about weekly consumptions of meat, fish, eggs, milk, and fruit because these items were not consumed on a daily basis. The 1-week intake of these food items was then divided by 7 to compute the average intake per day. We calculated total 24 hour intake of each nutrient using a spreadsheet program based on food composition tables.

Field physicians interviewed participants using a structured questionnaire, conducted a general examination, and obtained blood samples from each participant when they were visited in their homes. We have previously presented detailed information concerning storage and analysis of blood samples [6]. In brief, nonfasting blood samples were collected and stored in an ice chest in the field. Aliquots were prepared within 24 hours, frozen at -20 deg C in India, later transported to the United States on dry ice where they were stored at -70 deg C until laboratory analysis. Pacific Biometrics (Seattle, WA) conducted most serum and plasma analyses for the micronutrients and biochemical indicators, or in some instances arranged for them to be done at a different laboratory. Plasma

measurements included homocysteine, glutathione, cysteine, methionine, vitamin B6, retinol (Vitamin A), alpha-tocopherol (Vitamin E), alpha-carotene, beta-carotene, lycopene, lutein-zeaxanthin, and beta-cryptodextrin.

Serum measurements included glucose, cholesterol, vitamin B12, folate, transthyretin, and selenium.

Measurement of urinary arsenic

The urinary concentrations of arsenic were measured using hydride generation atomic absorption spectroscopy. In this technique, InAs, MMA, and DMA were reduced to the corresponding arsine in a batch reactor using sodium borohydride in 5 ml samples. The volatile reduction products (arsenic, methyl arsine, and dimethylarsine) were removed by sparging with helium. Entrained arsines were concentrated in a chromosorb-filled cryogenic trap in liquid nitrogen temperatures until all arsine-forming arsenic in the sample had reacted. The cryotrap was then allowed to warm, and the collected arsines were separated on the basis of differential volatilization. The separated volatile arsenic species were detected with a hydrogen microburner combustion cell to convert arsines to elemental arsenic. To prevent interference by other compounds, each urine sample was acidified with 2 M HCl and allowed to sit for at least 4 hours. Total arsenic was determined by flow injection analysis/atomic fluorescence spectrometry and the result was compared with the sum of the species detected. If a significant amount of arsenic remained undetected, additional digestion or assay for asenobetaine was performed. Detection limits for InAS, MMA, and DMA were 0.5, 1, and 2 micrograms/L respectively. Concentrations below the detection limit were set at one half the detection limit. The MMA and DMA measured in this study were in the pentavalent forms. The trivalent forms, MMA(III) and DMA(III), are rapidly oxidized to MMA(V) and DMA(V) during storage.

Demographic and social variables

We considered age, gender, type of housing and years of education as demographic and socioeconomic covariates. In India, socioeconomic status is commonly measured by the types of dwellings, which are correlated with household economic status [15]. In this study, we determined socioeconomic status by the dwelling type based on materials used to construct the house, and by educational attainment. We considered three classes of houses: pucca houses – built with high quality materials, eg bricks or concrete; semi-pucca houses – constructed partly with clay and bricks, and kacha – mud houses.

Statistical methods

We converted age into a categorical variable; we categorized educational attainment as follows: those who did not have formal education, those who studied till primary level (primary: about 4 years of formal education), those who attained high school but did not study beyond high school (secondary), and those who completed high school education and studied beyond (high school plus). Total urinary arsenic, percentages of inorganic arsenic in urine, percentages of MMA in urine, percentages of DMA in urine, and Body Mass Index (bmi) were categorized into quintiles. The status of skin lesions ("disease status") was based on the presence or absence of characteristic skin lesions attributed to caused by exposure to inorganic arsenic.

We cross-tabulated each categorical variable with indicators of methylation – percentages of inorganic arsenic (InAs%), MMA (MMA%) and DMA (DMA%) in urine and conducted tests for trends across the strata of each variable..

We analyzed 30 dietary factors and 16 blood level micronutrients. To remove outliers, we transformed all 46 dietary and micronutrient variables into their natural logarithms and removed all values that were 3 standard deviations below or above mean. The remaining values, retained for analysis, were then back transformed to their natural values and entered into further analyses.

We then regressed each indicator of methylation (urinary percentages of inoragnic arsenic, MMA, and DMA) on each dietary factor and blood micronutrient using a series of bivariate linear regressions. For each pair of bivariate linear regression, we calculated the standardized beta coefficient and the associated p-value.

We then selected a particular dietary factor or blood micronutrient to be included in a series of stepwise multivariate linear regressions as follows. For each pair of bivariate linear regression outlined in the above step, the standardized beta and its associated p-value was examined. For any particular dietary factor and serum micronutrient, if we found that the p-value associated with the beta coefficient was less than 0.05, we retained this dietary factor or serum micronutrient for further input to the series of multivariate reactions. We also calculated a correlation matrix of the selected dietary factors and serum micronutrients examine their collinearity with each other.

We then entered the selected dietary factors and the blood micronutrients in a series of multivariate linear regressions. The first outcome variable we considered was InAs%. The explanatory variables were entered in order of the magnitude, denoted by the absolute value of their standardized beta coefficient obtained during the bivariate regressions. The variables were added in a stepwise manner in decreasing order of beta coefficient values. We retained a variable if in the multivariate model, we found that the associated p-value for the variable was less than 0.05. We rejected a variable for the next step if p-value for the particular variable exceeded 0.05. We also added the following covariates into the multivariate model to deal with potential confounding: age in years (categorized), gender, education, housing status, total urinary arsenic, and presence or otherwise of skin lesions.

Results

In this population, about 75% people were aged between 20 and 59 years and about 63% were men (Table 1). Less than 5% of the population had high school or higher education, and about 14% lived in houses made of bricks. More than 50% of this population lived in kacha houses (houses constructed with clay). The median total urinary arsenic level was 128 microgram/L and the median values for urinary arsenic metabolites were 15.9%(InAs%), 6.6%(MMA%), 69.9%(DMA%) (Table 1).

Male gender and presence of skin lesions were associated with reduced output of InAs% and increased output of MMA% in urine, indicating increase in the first stpe of methylation to MMA, and decrease in the second to DMA.. Men had lower InAs% (men: 22.2%, women: 24.5%; p=0.05) and higher MMA% than women (men: 8.88, women: 7.02% ; p=0.01). Those who had skin lesions had lower InAs% (21.3% versus 24.3% for those with no skin lesions; p=0.03), and higher DMA% in urine (70.4% for those with skin lesions and 67.3% for those with no skin lesions). In summary, male gender and presence of arsenic-caused skin lesions were associated with increased methylation (Table 2).

From our bivariate linear regression models, we found nine dietary variables and three serum micronutrients had standardized beta coefficients with p-values lower than 0.05. These variables are described for each indicator of methylation in decreasing order of their standardized beta coefficients. For inorganic arsenic percentages, these were Serum Folate (beta = 0.392) with and Serum Selenium (beta = 0.219). For urinary MMA%, these were: Serum Folate (beta = -0.526), Dietary Animal Fat (beta = 0.440), Dietary Riboflavine (beta = 0.424), Dietary Animal Protein (beta = 0.298), Dietary Phosphate (beta = 0.297), Dietary Vitamin A (beta = 0.279), Serum Selenium (beta = -0.249), Dietary Calcium (beta = 0.245), Serum Homocysteine (beta = 0.234), Dietary Lysine (beta = 0.194), Dietary Fiber (0.191), and Dietary Thiamin (beta = 0.188). For DMA%, we found Serum Folate (beta = -0.263) and Serum Selenium (beta = -0.160) to have p-values less than

0.05 (Table 3).

In multivariate linear regression models, after controlling for the effects of age, gender, socioeconomic status, body mass index, arsenic exposure levels and presence of skin lesions, we found that Dietary Animal Fat and Dietary Phosphate were positively associated with MMA% and Serum Folate and Serum Selenium were negatively associated with MMA%. We also found Serum Folate and Serum Selenium positively associated with InAs%. When combined with different dietary variables and serum micronutrients, the standardized multivariate beta for Serum Folate varied between -0.363 to -0.263, indicating relative resilience of Serum Folate to the effects of other dietary variables and serum micronutrients. When combined with different dietary and serum micronutrients, the standardized multivariate beta for Dietary Animal Fat varied between 0.272 and 0.442, indicating relative resilience of Dietary Animal Fat to the effects of all other variables. In the model where Serum Folate and Dietary Animal Fat were put together, beta of Serum Folate was -0.305, or about 60% increment towards null, while that of Dietary Animal Fat was 0.442, indicating that even after adjustment for the effects of Serum Folate, the effect of Dietary Animal Fat was strong on MMA%. While Dietary Animal Fat was strongly correlated with Egg and Fish Fat, it had low correlation with Meat Fat. Egg fat, Fish fat and Meat derived Fat were not considered in the models. When Serum Folate and Serum Homocysteine were put in the same model, beta coefficients of both the variables were reduced; however, Serum Homocysteine had minimal loss of beta when put in multivariate models in combination with other dietary and micronutrients excluding Serum Folate. Serum Folate and Serum Homocysteine were moderately correlated with each other (correlation coefficient = -0.298). Serum Selenium was negatively correlated with MMA% but positively correlated with InAs%. The positive association of Serum Selenium persisted even after adjustment of all other variables including that of Serum Folate (Table 5)

Discussion

Increased dietary intake of Animal Fat was associated with increased MMA% in urine, while increased serum levels of Folate and Selenium were associated with decreased urinary MMA%. In addition, increased Serum Selenium was associated with increased urinary InAs%. When these findings were considered together, these tend to suggest that, increased dietary intake of Dietary Animal Fat may be associated with increased Arsenic Methylation with high proportional respresentation of MMA in urine, while high serum levels of Folate and Selenium would likely to reduce InAs conversion to MMA, resulting in reduced proportion of MMA and higher relative proportion of inorganic arsenic. High dietary intake of Animal Fat and Animal Protein were associated with increased urinary MMA%; dietary Animal Protein and Animal Fat were also highly correlated with each other ($r = 0.611$; $p = 0.03$). When MMA% was regressed on Dietary Animal Protein and Dietary Animal Fat together in the multivariate regression model, the effect of dietary Animal Fat persisted while that of dietary Animal Protein was diminished, indicating that even after adjusting for the effects of other dietary factors, dietary intake of Animal Fat had an indepedent association with a methylation state that increased urinary MMA%.

This was a large population based study ($N = 406$) conducted in rural West Bengal among a population that was exposed to inorganic arsenic in groundwater exceeding 50 micrograms/Liter. The field investigators estimated the dietary factors in the field, and at the time of conducting the diet survey, they were not aware of the methylation status of the participants; in addition, estimation of urinary levels of arsenic metabolites and serum micronutrients were done separately, thus removing any possible observation or measurement bias. In addition to dietary Animal Fat, Animal Protein, serum Folate, and serum Selenium, other dietary variables and micronutreints considered for the multivariate models were: dietary Fiber, Phosphate, Riboflavine, Vitamin A, Lysine, Calcium, Thiamine, and serum Homocysteine. We tested the effects of all these other dietary factors and serum levels of micronutrients in a series of multivariate models. In these models, we added

age, gender, socioeconomic status, body mass index, total urinary arsenic (as a measure of arsenic exposure) and skin lesions as additional covariates. Serum Homocysteine dropped out of multivariate models when added in combination with dietary Animal Fat and Serum Folate levels. Presence of these associations in the context of large sample sizes, objectivity in the measurements and blinding of the investigators as to the status of methylation and serum levels of micronutrients, and persistence of effects in multivariate models suggest that the observed associations between MMA%, dietary Animal Fat, Serum Selenium, and Serum Folate levels, and associations between urinary InAs%, and serum Selenium, were real.

In vivo methylation of inorganic arsenic uses S-adenosyl methionine (SAM) as the methyl group donor, and the end results of all SAM dependent methylation reactions are methylated arsenic products and the SAH (s-adenosylhomocysteine). SAH is then converted to homocysteine in a rapidly reversible reaction. Even mild increments in plasma homocysteine levels are associated with linear increments in SAH levels. SAH also act as rate limiting inhibitor of the methylation, by tightly binding to methyltransferases and is only removed with downstream removal of homocysteine, which occurs with folate supplementation or with increasing folate levels. We found that Homocysteine (serum) was positively correlated with MMA% (beta = 0.234; p = 0.04). Homocysteine was also negatively correlated with serum Folate (r = -0.298, p = 0.01). However, when both Homocysteine (serum) and Folate (serum) were included in the same regression model, the coefficient of Folate (serum) remained unchanged while that of Homocysteine (serum) reduced (bivariate beta = 0.19, p= 0.01). In a large cross-sectional survey in a comparable Bangladeshi population (N = 1650), Gamble reported negative association between serum Folate with MMA% (r = -0.12; p = 0.04) and positive association between serum Homocysteine and urinary MMA% (r = 0.21; p < 0.001). However they did not analyze the data adjusting for each other.whne looking at MMA%.

The association between high dietary intake of Animal Fat and high urinary MMA% was

surprising before this was not reported before, and we did not find any prior published research that explained any biological role of dietary animal fat in affecting arsenic methylation. When we regressed urinary MMA% on dietary Animal Fat and dietary Animal Protein together in the multivariate regression model, beta coefficient of dietary Animal Fat remained unchanged from the bivariate beta coefficient, while that of the dietary Animal Protein reduced. Thus, the effect of dietary Animal Fat on urinary MMA% was an independent effect that persisted even after adjustment for the effects of all other variables. There has been no prior report of the association between dietary Animal Fat and increased MMA%, and ours is the first report of this association. While the biological significance or mechanism by which dietary intake of Animal Fat may impact urinary MMA% is unclear, we also know that in this population, fish intake was the major contributor to the dietary Animal Fat concentration (data not shown).

The findings of this study need be interpreted in the light of its several limitations. First, although we found associations between dietary Animal Fat, serum Folate, and serum Selenium with urinary MMA% and serum Selenium with InAs% , the magnitude of these associations were weak, based on standardized beta coefficients as measure of the slope of the linear association, indicating at best modest effects. Second, since this was a cross-sectional survey, information on the dietary factors were collected at the same time as blood was sampled for estimation of serum micronutrients and urine samples were collected for estimation of urinary InAs%, MMA%, and DMA%. Because of the complex inter-relationships between arsenic methylation reactions and micronutrients including Folate (serum), and Homocysteine (serum), involving transmission of methyl groups, it could not be ascertained only on the basis of a cross-sectional study whether the changes in either dietary intake of Animal Fat, or levels of Homocysteine, Folate (serum), or serum Selenium preceded the changes in the levels of urinary arsenic metabolites. Third, we obtained data on dietary factors using a diet based food frequency questionnaire based on 24 hours recall, with the tacit assumption that the dietary patterns of people in this population remained unchanged for a long

time. While this assumption may have been a valid assumption in view of this stable, rural population, this assumption may not be tenable in other populations that have more variations in dietary intake of items over time.

In summary, while this study had several limitations, the findings suggested that serum Folate, serum Selenium, and dietary Animal Fat may be important independent factors that explained variability of urinary MMA% and InAs% and thereby overall methylation of arsenic among individuals who lived in high exposure areas. The findings that dietary Animal Fat may be linked to Arsenic methylation was unexpected, particularly in view of the observations that although majority of the Animal Fat intake could be explained by dietary Fish Fat intake rather than dietary Meat intake, neither Fish Fat nor Meat Fat had associations with Arsenic Methylation. These need to be further characterized in future studies linking dietary patterns contributing to arsenic toxicity among populations exposed to high Arsenic.

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CHAPTER 5: METHYLATION AND SKIN LESIONS

Abstract

Background. – Worldwide, exposure to high concentrations of inorganic arsenic through drinking water accounts for considerable mortality and morbidity. It is believed that increased methylation of arsenic, leading to generation of MMA(III), increases the risk of arsenic toxicity. While the role of methylation has been studied in the context of arsenic toxicity, relatively little is known about the impact of different dietary intakes and blood level micronutrients on arsenic methylation. Here, we report the impact of dietary variables and serum micronutrients on arsenic methylation in a population known to be exposed to inorganic arsenic.

Methods. – A case control study on the effects of dietary and serum level variables on arsenic-caused skin lesions was nested within a larger cross sectional survey. Information on dietary variables was obtained through a diet survey questionnaire, and information on blood level micronutrients was obtained from sampling during the cross sectional survey. Methylation capacity was measured by percent excretion of inorganic arsenic, monomethyl arsonous and arsonic acids (MMA), dimethylarsinic acids (DMA) in urine and by calculating the MMA:DMA ratio. Adjustments for dietary calorie intakes were done for dietary variables by dividing the dietary variables by calorie intake. Levels of calorie-adjusted dietary intake and serum micronutrients were calculated by calculating quintiles. Mean values of the indicators of arsenic methylation for the lowest and highest quintiles of dietary and micronutrient levels were used to assess the crude association between dietary variables, serum micronutrients and methylation capacity. Multivariate linear regression models were used where indicators of methylation were regressed on dietary and serummicronutrients after adjusint for the effects of age in years, gender, housing status, educational attainment, total urinary excreted arsenic. The beta coefficients were used to identify

the effects of increment or decrement of dietary variables on serum micronutrients on methylation status.

Results. – Older age was associated with increased MMA%, and MMA:DMA ratio; female gender was associated with high InAs%,but low MMA% and low MMA:DMA ratio. No effect was observed for educational attainment, housing status, or total arsenic excreted in urine.

Compared to those in the lowest quintiles of calorie adjusted dietary intakes of animal protein, animal fat, calcium, phosphate, Vitamin A, Riboflavin, Lysine and Tryptophan, those who were in the highest quintiles of these dietary intakes had high MMA% and MMA:DMA ratios. After adjustment for age, gender, socioeconomic status, and total urinary arsenic, high MMA percentage was positively associated with animal protein intake (beta = 0.16), animal fat (beta = 0.49), calcium (beta = 0.004), phosphate (beta = 0.007), vitamin A (beta = 0.026), Riboflavin (beta = 5.5), and Lysine (beta = 0.003). Dietary intakes of animal protein (b = 0.34), animal fat(b = 0.88), calcium (b = 0.008), phosphates (b = 0.011), Vitamin A (b = 0.047), Riboflavin (b = 11.1) and lysine (b = 0.005) were positively associated with MMA:DMA ratio.

Compared to those in the lowest quintiles of blood levels of glucose, folates, and selenium, those who were in the highest quintiles had high InAs%; those in the highest quintiles of selenium and glucose had low MMA%, low DMA%, and low MMA:DMA ratios. Compared to those in the lowest quintiles of blood levels of retinol, Vitamin B6, and methionine, those who were in the highest quintiles had higher MMA% and MMA:DMA ratio. Those who were in the highest quintiles of homocysteine, had high MMA%. After adjusting for the effects of age, gender, socioeconomic status, and total urinary arsenic, blood levels of retinol was negatively associated with InAs% (b = -0.15), but positively associated with MMA% (b = 0.06) and MMA:DMA ratio (b = 0.09). In the multivariate model, blood selenium was negatively associated with MMA% (b = -1.34), and Vitamin B6 was positively associated with MMA% (b = 0.016), and MMA:DMA ratio

(b = 0.029).

Discussion. – In summary, high calorie adjusted dietary intakes of animal fat, animal protein, Riboflavin, Vitamin A, calcium and phosphates were associated with increased methylation, while high blood levels of retinol and Vitamin B6 were associated with increased methylation. High blood levels of selenium was associated with low methylation,indicated by low MMA levels. These indicate that diet and micronutrients play potentially important roles in the arsenic metabolism and warrant further studies.

Introduction

Worldwide, millions of people are exposed to high concentrations of inorganic arsenic in their drinking water. In the Indian subcontinent alone, about 150 million people in Bangladesh and the adjacent areas of the West Bengal state of India in the Ganges delta are exposed to very high concentrations of inorganic arsenic in their drinking water [Error: Reference source not found]. Exposure to high concentrations of inorganic arsenic is associated with different clinical manifestations ranging from skin lesions through systemic illnesses (hypertension, diabetes mellitus) to internal cancers (cancers of lung, and urinary bladder). Following entry to human body, arsenic undergoes sequential processes of reduction and methylation reactions where the methyl groups are donated by S-adenosyl methionine (SAM), resulting in the process of formation of different arsenic species and compounds — As(V), As(III), monomethyl arsonous acid (MMA(III)) and MMA(V), and dimethyl arsinic acids - DMA(III) and DMA(V) — the roman numerals in the parentheses indicating their respective valency states [Error: Reference source not found]. Till recently, methylation was believed to be a process of detoxification of arsenic; however, current evidence following isolation and characterization of MMA(III) indicate that methylation may not be a detoxification process; in fact, methylation can actually increase susceptibility to several arsenic-caused health effects, in particular, arsenic-caused cancers of skin and urinary bladder [18]. It is

believed that increased methylation process increases cell-wide DNA hypermethylation, leading to deficiency of DNA repairs and inactivation of tumor suppressor proteins. The combined effects of altered methylation patterns in the tissues lead to development of tumor formation [Error: Reference source not found].Inorganic arsenic, after entry into body, undergoes sequential reduction and methylation processes that lead to the formation of monomethyl arsonous acids and dimethylarsinic acids — these metabolites are then excreted by the urine.

Macronutrients and micronutrients have played important roles in the pathogenesis of chronic arsenic toxicity. Earlier studies have shown that people with arsenic-caused skin lesions have low dietary intakes of animal protein, calcium, and dietary fibers; studies in Taiwan with arsenic caused peripheral vascular lesions have suggested that people with arsenic-caused health outcomes have low levels of beta carotene. Low levels of selenium have been implicated in enhancing susceptibility to arsenic toxicity [12, Error: Reference source not found, 19].

There is limited evidence on the inter-relationship between diet, micronutrients, methylation of arsenic and clinical outcomes as a result of arsenic exposure. However, arsenic methylation uses methionine and homocysteine directly in the metabolic pathway, and several micronutrients may be involved as co-factors for the enzymatic reactions during the methylation process. An earlier study has suggested that people whose diets are deficient in protein, iron, zinc and niacin show higher excretion of MMA and are more susceptible to arsenic-caused cancers [18]. However, little is known to the extent micronutrients and dietary factor affect arsenic methylation patterns based on human epidemiological studies. The purpose of this paper is to present data on the relationship between diet, micronutrients and indicators of arsenic methylation.

Methods

We conducted a population based case control study nested within a larger cross-sectional survey of an arsenic-endemic region, the North 24 Parganas district, of the state of West Bengal in India. Below, we outline the population, exposure variables, comparison groups, and measurements of the outcome.

Base Population. — We targeted two areas within the North 24 Parganas district, West Bengal, for this survey. We selected the first area because high levels of arsenic were detected in some, but not all, of the shallow tubewells as determined in a prior study [Error: Reference source not found]. The second area included the remaining part of the district where people used shallow tubewells for the source of their drinking water. The total population of the two araes combined was 150,457. A total of 7818 participated in the study, and we obtained water arsenic levels for 7683 individuals (98.3%). These 7683 individuals (4093 females and 3590 males) consitute the base population for this study.

In this geographical region, the high exopsure region (where the aveargе arsenic level in groundwater >= 50 micrograms/l) included 25 villages. The study team went to the center of each village and selected the most convenient group of houses to commence sampling. The team invited each member of the household present at the time of the interview to participate. They adminitered an interview and conducted medical examination. Sampling continued house-to-house in a village till the team recruited between 50–150 participants.

The low exposure region included 32 villages within 16 amdinistrative blocks. We restricted the sampling in this region to villages with more than 100 houses. One or more villages were selected at random from each of the 16 blocks depending on the population size. While we selected only one village for sampling from a small block, if the block was large, we also selected two to three villages. As with the high exposure villages, the study team went to the center of each village, and

selected the most convenient groups of houses to commence sampling. Here, we invited residents of every fourth house to participate.

Eligible population. – The arsenic levels in the groundwater ranged from nondetectable through 3400 micrograms/liter (mean = 185 micrograms/liter; standard deviation = 290). Because we were interested to study effects at low doses, we based this case control study on survey participants living in the 21 villages whose primary drinking water sources contained < 500 micrograms/liter of inorganic arsenic. We found 4185 individuals (54.5%) who fulfilled these criteria (N = 4185; 2160 females and 2025 males).

Participant population. – We labelled cases as all individuals from the cross sectional survey who were diagnosed with arsenic-caused skin lesions (described in the next section) and whose main water source contained less than 500 micrograms/liter of arsenic. From 4185 individuals who were eligible to participate in the study, the participant population was 265 individuals with skin lesions (6.3%). Of the 265 identified cases, 174 had pigmentation changes, 15 had keratoses and 76 had both types of lesions. Hyperpigmentation is marked by raindrop-shaped discolored spots, diffuse dark brown spots, or diffuse darkening of the skin on the limbs and trunk. Simple keratoses usually appear as bilateral thickening of the palms and soles. In nodular keratosis, small protrusions emerge on the palms and soles, and also occasionally on the dorsum of the hands and feet, or on the legs. We selected controls from survey participants who did not have skin keratoses or hyperpigmentation when seen during the 1995–1996 survey, and whose main tube well-water source, like the cases contained < 500 micrograms/liter of arsenic. For each case, we selected one control matched on age (plus or minus 5 years) and sex randomly from all eligible non-cases. We selected replacement controls for controls who had died, could not be located or did not wish to participate. Of the 530 initial seleced individuals (265 cases and 265 controls), 15 cases were not available to participate, 16 individuals (6 cases and 10 controls) moved outside the study region, 54 (26 cases and 28 controls) could not be located, and 40 individuals (26 cases and 14 controls) were

either too ill to participate or died or refused to participate. Thus, we recruited 405 individuals (192 cases and 213 controls) for this study.

Measurement of urinary arsenic species. – Two to three urine samples were collected from each participant over one-year period. Subjects were given screw-top containers and asked to give a midstream sample of the first morning void. Samples were then transported on ice to the field laboratory each day where they were kept frozen at -20 degree C. Urine samples were transported to the University of Washington, Seattle, for analysis.

The urinary concentrations of arsenic were measured using hydride generation atomic absorption spectroscopy. In this technique, InAs, MMA, and DMA were reduced to the corresponding arsine in a batch reactor using sodium borohydride in 5 ml samples. The volatile reduction products (arsenic, methyl arsine, and dimethylarsine) were removed by sparging with helium. Entrained arsines were concentrated in a chromosorb-filled cryogenic trap in liquid nitrogen temperatures until all arsine-forming arsenic in the sample had reacted. The cryotrap was then allowed to warm, and the collected arsines were separated on the basis of differential volatilization. The separated volatile arsenic species were detected with a hydrogen microburner combustion cell to convert arsines to elemental arsenic. To prevent interference by certain compounds, each urine sample was acidified with 2 M HCl and allowed to sit for at least 4 hours. Total arsenic was determined by flow injection analysis/atomic fluorescence spectrometry and the result was compared with the sum of the species detected. If a significant amount of arsenic remained undetected, additional digestion or assay for asenobetaine was performed. Detection limits for InAS, MMA, and DMA were 0.5, 1, and 2 micrograms/L respectively. Concentrations below the detection limit were set at one half the detection limit. The MMA and DMA measured in this study were in the pentavalent forms. The trivalent forms, MMA(III) and DMA(III), are rapidly oxidized to MMA(V) and DMA(V) during storage [7].

Ascertainment of social and demographic variables. – In India, socioeconomic status is commonly measured by the types of dwellings, which, in turn are correlated with household economic status [15]. Hence, in this study, socioeconomic status was determined by two parameters: dwelling type based on materials used to construct the house and educational attainment. Three different dwelling types were described: pucca, indicating houses that were built with high quality materials, eg bricks or concrete, those constructed partially with clay and bricks (semi-pucca), and those that were made mainly of clay (kacha). The other indicator for socioeconomic indicator was educational attainment. In our study, we classified educational attainment in the following categories: no formal education, the respondent only attended primary schools and then dropped out (primary education), the respondent attended secondary schools (ie, had 10 years of formal education), and the respondent finished high school and achieved higher education (high school and above).

Assessment of dietary variables. – Weight and height were measured, and Body Mass Index was calculated. Food intake was ascertained by a detailed questionnaire based on 24-hour recall. The "senior" woman, who in this population, directs preparing food for the family, was interveiwed. Raw food materials were weighed whenever the participating woman was unable to state the actual weight of the food used. To estimate the amount and volume of food consumed, plates and cups of various sizes were shown to the participating woman during the interview. Dish volumes were standard amounts listed by India's National Institute of Nutrition for use in diet surveys.

The participating woman was questioned about each meal, from the previous day's lunch through the breakfast on the day of the interview. All food items used to prepare each meal were noted, and their weights in grams were recorded in the questionnaire. The volume of each cooked food was assessed by questioning the senior woman using standard cups and plates. From the total cooked food, the portion given to the individual participant was also assessed by cups and plates exhibited to the woman. Standard size spoons were used to assess the intake of sugar and oil.

Participants who worked in the fields often carried food from home. If not, then the participant was questioned about purchased food items. Each participant was also questioned directly about his or her food likes and dislikes and asked to briefly confirm what was reported by the woman of the house. Questions were asked about the weekly consumption of meat, fish, eggs, milk, and fruit because these items were not consumed daily. A one-week intake of these food items as then divided by 7 to calculate the daily average intake. Individual intake in terms of raw food item (rice, legumes, potato) was calculated by the formula $F=(P/Q)*R$ where F was individual intake of a raw food item by the study participant from a particular preparation, P was the amount in grams of the total raw food ingredient, F, usd for cooking this preparation, and Q was the total volume in milliliters of cooked food preparation consumed by the study participant.

The total intake (24-hourly) of each nutrient (carbohydrate, protein, vitamins), and calorie consumption were calculated using a spreadsheet program. A detailed database was set up for the nutrient consumption per 100 g raw food items.

The protein, fat, carbohydrate, energy, fiber, mineral (calcium, phosphorus, iron, and zinc), and vitamin (thiamin, riboflavin, niacin, vitamin B6, folate, carotene, retinol, and vitamin C composition of raw foods were obtained from information published by the National Institute of Nutrition, India. As in other countries, selenium intake in the diet is variable depending on the soil where food is grown. Hence, it was not possible to asess selenium intake in this study.

Food composition (nutrition values) for ready-to-eat items (biscuits, health beverages, skimmed milk) were obtained from the package information. Food composition of items not typically prepared in homes was averaged from information obtained from the owners of five shops. Vitamin supplements are not used in the study population.

Ascertainment of micronutrients. – Field physicians interviewed participants using a structured quesionnaire, conducted general medical examination, and obtained blood samples the same day

participants were located. Informed consents were obtained from all participants. Nonfasting blood samples were obtained on 180 cases and 196 controls. Blood samples were stored in a covered chest (0 degree centigrade) in the field to prevent degradation of light and temperature sensitive compounds. Approximately half (58%) of the blood samples were centrifuged and aliquoted into serum and plasma samples the same day, while the rest were refrigerated overnight (4 degree centigrade) and aliquoted the next day. Aliquots were kept frozen at -20 degree Centigrades in India, delivered on dry ice, nd stored at -70 degree Centigrade in the US, until analysis.

Laboratory methods. – Pacific Biometrics (Seattle, WA) conducted serum and plasma analyses for the micronutrients and biochemical indicators unless otherwise noted. Assays were conducted blind to the case or control status of each sample. Methods used for each nutrient are briefly presented. Plasma indicators measured include homocysteine, glutathione, cysteine, methionine, and vitamin B6. Plasma levels of retinol (Vitamin A), alpha-tocopherol (Vitamin E), alpha-carotene, beta-carotene, lycopene, lutein-zeaxanthin, and beta-cryptodextrin were also measured. Plasma thiols (homocysteine, glutathione, and cysteine) were measured by high performance liquild chromatography (HPLC) using an internal standard and monobromobimane derivatization. Plasma methionine was measured by amino acid analyzer (Beckman 6300 Amino Acid Analyzer) using a cation-ion exchange column at the Scientific Research Consortium, Inc. Vitamin B6 (Pyridoxal phosphate) was determined by HPLC and fluorescence detection involving precolumn derivatization of plasma vitamers with sodium bisulfite. Plasma retinol, alpha-tocopherol, beta-carotene, lycopene, lutein/zeaxanthin, andbeta-cryptodextrin were analyzed by isocratic reverse-phase HPLC after addition of internal standards and total lipid extraction.

Serum measurements include glucose, cholesterol, vitamin B12, folate, transthyretin, and selenium. Glucose was assayed using an automated version of the Barthelmai and Czok gluose assay (the hexokinase/glucose-6-phosphate dehydrogenase). Total serum cholesterol was quantified with the CDC-standardized Trinder end-point reaction in an automated chemistry analyzer. Levels

of vitamin B12 and folate were analyzed by CEDIA assays on the Hitachi 911 using Roche reagents. Transthyretin levels were determined by immunopreciptin analysis. Sample transthyretin concentrations were calculated from a standard curve established for each assay batch. Selenium was measured by graphite furnace atomic absorption spectrophotometry at the Nutrition Research Laboratory in the Department of Laboratory Medicine t the UUniversity of Washington or by inductively coupled plasma mass spectrometry at the Associated Regional and University Pathologists trace mineral laboratory at the University of Utah Health Science Center (Salt Lake City, UT).

Statistical data analysis. –

Dietary variables were adjusted for calorie intakes by dividing the dietary intakes by calorie intakes. Quintiles of calorie-adjusted dietary intakes and levels of serum micronutrients were used for further analysis as explanatory variables. The following outcome variables were used: percentages of excreted inorganic arsenic (InAs%), monomethyl arsonous and arsonic acids (MMA %), dimethyl arsinic acids (DMA%) and MMA:DMA ratio. Covariates included age in years, gender, levels of education, types of housing, and total arsenic species in urine.

One-way ANOVA (ANalysis Of VAriance) was used to test the mean differences in InAs%, MMA%, DMA%, and MMA:DMA ratios against age in years, gender, levels of education, types of housing, and quintiles of total arsenic species in urine. One-way ANOVA was also used to test the mean differences between highest and lowest quintiles of dietary intakes and serum micronutrients.

Multivariate models. – Associations between dietary intakes, serum micronutrients and indicators of methylation were studied by several bi-, and multivariate linear regression models. In each of these models, the outcome variable was an indicator of methylation (InAs%, MMA%, DMA%, or MMA:DMA ratio), and the exposure variable was an item of dietary intake, or a serum micronutrient. For multivariate linear regression models, following co-variates were included: age

in years (categorized), gender, levels of education, types of housing, and quintiles of total urinary arsenic species. The standardized regression coefficients and model chi-squares were compared between the bivariate and multivariate models to assess the impact of the covariates in the association between dietary, serum micronutrient, and outcome variables.

Results

More than 90% of the participants were less than 60 years old, and about 38% were less than 30 years, and about 64% were male. In this population, about 30% had no formal education, and less than 5% population had completed high school and attained higher education beyond high school, and about 52% lived in kacha houses. The median percentages of excreted inorganic arsenic, MMA, and DMA in urine were respectively 20.7, 8.56, and 74.8, while the median MMA:DMA ratio was 12.2 indicating high MMA output relative to DMA excretion (Table 1).

Older age groups and male gender was associated with higher MMA% and high MMA:DMA ratios, while no statistically significant association was observed between educational attainment, type of housing and total excreted arsenic concentration in urine (Table 2).

Low calorie-adjusted dietary intakes of niacin, but high calorie-adjusted dietary intakes of animal protein, animal fat, calcium, phosphate, Vitamin A, Riboflavin, Lysine, Leucinea and Isoleucine were associated with increased MMA excretion. Higher calorie adjusted dietary intakes of Vitamin A was associated with high DMA excretion (Table 3).

Compared to those in the lowest quintiles of blood levels of glucose, folate, and selenium, those who were in the highest quintiles had higher percentage of inorganic arsenic in their urine. Glucose, folate, and selenium were also associated with decreased MMA percentages, and decreased MMA:DMA ratio. In summary, blood glucose, folate, and selenium were associated with increased unchanged inorganic arsenic excretion but reduced MMA percents and reduced MMA:DMA. These indicate that these three micronutrients were associated with low methylation per se. On the other hand, homocysteine, retinol, vitamin B6, and methionine were associated with increased MMA excretion, and increased MMA:DMA ratio, indicating that these were associated with high methylation (Table 4).

These associations were tested in a series of bi- and multi-variate linear regressions where

indicators of methylations were regressed on the dietary and serum micronutrients. In the multivariable models, the following co-variates were added to control for their effects: age in years, gender as indicators of their demographic profiles, and educational attainment and housing conditions as measures of their socioeconomic status, and total excreted arsenic content in urine (measure of arsenic exposure). Standardized betas were used to test the strength and direction of association between diet, micronutrients, and indicators of methylation.

In the bivariate analysis, the following dietary variables were found to be statistically significantly associated with MMA percentages (in decreasing order of their betas): riboflavin, niacin, animal fat, animal protein, vitamin A, phosphates, calcium, and lysine. All of these except niacin, were found to increase the proportion of MMA. These effects persisted even after adjustment for the effects of demographic, socioeconomic factors and the effects of arsenic exposure. Dietary niacin was found to be negatively associated with MMA proportion, indicating that it was associated with reduced methylation (Table 3).

Glucose, folate, and selenium were associated with increased inorganic arsenic excretion percentages and reduced MMA percentage excretion, while homocysteine and retinol were associated with reduced inorganic arsenic percentages and increased MMA percentages. These figures suggested that in crude estimates, glucose, folate, and selenium were associated with reduced methylation and homocysteine and retinol were associated with increased methylation (Table 4).

These associations were further tested with a series of bivariate and multivariate linear regressions. The standardized betas obtained from these linear regression models are shown in Table 5 for the dietary variables, and shown in Table 6 for the serum micronutrients. For inorganic arsenic percentage, no dietary variable was found to have statisticially significant association. The following dietary variables were found to have statistically significant association with MMA%:

animal protein, animal fat, phosphates, riboflavin, lysine, leucine, and isoleucine. For MMA%, unadjusted standardized beta eestimate for animal protein was 0.13; this meant that for every 3.5 g increment of calorie adjusted dietary intake of animal protein, MMA% in urine would increase by about 0.46, and after adjustment for the effects of age, gender, housing conditions, education, and urine arsenic levels, for every 3.5 g increment in calorie adjusted dietary intake of animal protein, MMA% in urine would increase by about 0.56, indicating that adjustment for demographic, socioeconomic, or exposure variables had very little effect on the association between dietary intake of animal protein and methylation (measured by MMA% output).

As for blood micronutrients, selenium was negatively associated with increased MMA%, while retinol and Vitamin B6 were positively associated with increased MMA%, indicating association of selenium with reduced methylation and that for retinol and Vitamin B6 with increased methylation capacities. After adjustment for the effects of demographic, socioeconomic, and exposure level factors, 0.4 unit increment in serum selenium levels were associated with 0.56% reduction in MMA % in urine. In comparison, after adjustment for demographic, socioeconomic and arsenic exposure factors, for every 10 microgram increment in serum retinol level, the MMA% output in urine would increase by about 0.6%, and for every 45 microgram/L increment in the levels of Vitamin B6, the MMA% in urine would go up by about 0.6%.

Discussion

To summarize the key findings of this study, we found that several dietary elements and serum level micronutrients were associated with either increased or decreased methylation, marked by the output of the percent MMA in urine. High dietary intakes of animal protein, animal fat, calcium, phosphates, Vitamin A, Riboflavin, Lysine and isoleucine, serum retinol and Vitamin B6 were associated with increased methylation (marked by increased MMA%), while increasing levels of serum selenium was associated with reduced arsenic methylation. These associations persisted even after adjustment for the effects of demographic and socioeconomic variables (age, gender, education, housing), and arsenic exposure marked by total arsenic levels in urine.

This study was based on a cross sectional survey of over 7600 individuals within which a case control study was nested (N = 406). Information about dietary variables was obtained by a dietician and the measurement of the serum micronutrients and indicators of methylation was done at different places so that there remained no possibility of these results being influenced by either observer or other forms of bias. Further, in these analyses, demographic (age, and gender), socioeconomic (types of housing, education), and arsenic exposure (total urinary arsenic) were adjusted for in the analyses to control for possible effects of confounding factors.

For dietary Riboflavin, Phosphates and Vitamin A, between the first (Q1) and highest quintiles (Q5) of dietary intakes, Inorganic arsenic%, and MMA% changed by more than 20% of their baseline values; similarly, for serum selenium, the change in inorganic arsenic and MMA% was about 20%, indicating that these dietary variables and micronutrients had strong effects on methylation (Tables 3 and 4). Additionally, dietary Riboflavin and serum selenium had high beta coefficients of linear regressions in multivariate models, indicating that dietary Riboflavin and Selenium had strong dose-response relationship as well.

Earlier studies have shown association between arsenic methylation and arsenic caused skin and

bladder cancer, and association between low dietary intakes of proteins, phosphates, calcium and increased likelihood of skin lesions. However, in contrast to dietary elements, no specific association between micronutrients and skin lesions were reported.

Conclusion

Arsenic methylation is a complex phenomenon with many regulatory steps involved. Some dietary elements and serum micronutrients may increase the methylation process, while others reduce them.

Table 1: Distribution of demographic, socio-economic, indicators of methylation, and total arsenic in urine for cases and controls

Variable	Category	Cases	%	Controls	%	Sig
Age in years						Matched
	<20	34	45.9	40	54.1	
	20-39	89	49.1	93	50.8	
	40-59	54	46.9	61	53.1	
	>60	15	44.1	19	55.8	
	Total	192		213		
Gender						Matched
	Female	73	48.9	76	51.1	
	Male	119	46.5	137	53.5	
	Total	192	95.5	213	100	
Housing						0.40
	Kacha	99	47.4	110	52.6	
	Pucca	30	51.7	28	48.3	
	Semi-pucca	63	45.7	75	54.4	
	Total	192		213		
Education						0.14
	No formal	59	50.4	58	49.5	
	Primary	97	48.5	103	51.5	
	Secondary	27	39.7	41	60.3	
	High School plus	9	45	11	55	
	Total	192		213		
InAs%						0.03
	>34	33	44	42	56	
	22.2 – 34	30	41.7	42	58.3	
	16.8 – 22.1	33	44	42	56.00	
	12.2 – 16.7	43	50.5	42	49.5	
	< 12.2	53	56.4	41	43.6	
	Total	192		213		

MMA%						0.30
	<4.26	28	40.6	42	59.4	
	4.26–6.57	47	52.8	43	47.2	
	6.58–8.37	35	45.5	43	54.5	
	8.38–11.2	43	50.6	43	49.4	
	>11.2	32	47.5	42	52.5	
	Total	192		213		
DMA%						0.21
	< 58.3	35	46.1	42	53.9	
	58.3–67.5	27	39.1	43	60.9	
	67.6–74.1	42	50	43	50	
	74.2–79.1	33	44	43	56	
	>79.1	55	56.7	42	43.3	
	Total	192		213		
MMA:DMA Ratio						0.21
	< 0.06	30	42.3	42	57.8	
	0.06–0.09	48	53.3	43	46.7	
	0.10–0.12	36	46.2	43	53.9	
	0.13–0.16	41	49.4	43	50.6	
	> 0.16	37	45.5	42	54.5	
	Total	192		213		
Total urine arsenic						0.02
	< 11.5	16	27.3	41	72.7	
	11.5–30.2	41	48.8	43	51.2	
	30.3–60.2	52	54.3	43	45.7	
	60.3–119	39	47.5	43	52.5	
	> 119	45	51.2	43	48.8	
	Total	192		213		

Table 2: Mean and interquartile ranges for InAs%, MMA%, DMA%, MMA:DMA ratios, total urinary arsenic, peak and average tubewell water arsenic concentrations.

	Cases			Conttrols		
Variable	Mean	Q1	Q3	Mean	Q1	Q3
InAs%	16.8	11.2	26.4	18.7	13.8	29.3
MMA%	7.59	5.29	10.5	7.48	4.96	10.3
DMA%	73.3	64.4	80.5	71.9	62.1	77.8
MMA:DMA ratio	0.11	0.07	0.15	0.11	0.08	0.15
Total urinary arsenic	53.6	26.3	118	43.2	14.4	93.7
Tubewell peak water conc	325	211	405	180	45	260
Tubewell av water conc	179	86	233	83	9	133

Table 3. Crude odds ratio between case control status and selected explanatory variables (adjusted for the effects of age and gender).

Variable	Category	Odds Ratio	95% Confidence Interval	
			Lower limit	Upper limit
InAs%				
	> 34	1.00		
	22.2–34	0.90	0.46	1.74
	16.8–22.1	0.99	0.51	1.90
	12.2–16.7	1.30	0.70	2.44
	< 12.2	1.66	0.90	3.07
MMA%				
	< 4.26	1.00		
	4.26–6.57	1.65	0.87	3.12
	6.58–8.37	1.25	0.65	2.43

	8.38–11.2	1.54	0.81	2.94
	>11.2	1.38	0.71	2.70
DMA%				
	< 58.3	1.00		
	58.3–67.5	0.76	0.39	1.47
	67.6–74.1	1.18	0.63	2.19
	74.2–79.1	0.92	0.49	1.76
	>79.1	1.53	0.84	2.80
MMA:DMA Ratio				
	< 0.06	1.00		
	0.06–0.09	1.62	0.86	3.06
	0.10–0.12	1.21	0.63	2.32
	0.13–0.16	1.39	0.73	2.65
	> 0.16	1.21	0.62	2.38

Table 4. Odds ratios adjusted for the effects of age, gender, housing and educational attainment. The estimates for age, gender, housing and education are not shown here.

Variable	**Category**	**Odds Ratio**	**95% confidence interval**	
			Lower limit	**Upper Limit**
InAs%				
	> 34	1.00		
	22.2–34	0.89	0.46	1.73
	16.8–22.2	0.93	0.48	1.80
	12.2–16.8	1.18	0.62	2.24

	< 12.2	1.51	0.81	2.83
MMA%				
	< 4.26	1.00		
	4.26–6.57	1.62	0.85	3.08
	6.57–8.37	1.17	0.60	2.29
	8.37–11.2	1.37	0.70	2.68
	> 11.2	1.24	0.62	2.44
DMA%				
	< 58.3	1.00		
	58.3–67.5	0.76	0.39	1.47
	67.5–74.1	1.08	0.57	2.05
	74.1–79.1	0.86	0.45	1.65
	> 79.1	1.43	0.77	2.64
MMA:DMA Ratio				
	< 0.06	1.00		
	0.06–0.09	1.55	0.81	2.96
	0.10–0.12	1.16	0.60	2.26
	0.13–0.16	1.28	0.66	2.49
	> 0.16	1.11	0.56	2.21

Table 5. Summary of the previous research on association between skin lesions and arsenic methylations

Author, Year	Study Type	Outcomes	Effect estimates
Hsueh, 1997	Cross-sectional	Skin cancers	MMA% <=26.7:8.35 (1.07–65.0) MMA% > 26.7: 23.9 (2.55–255)

Yu, 2000	Case control	Skin lesions	MMA%: 5.5 (1.22–34.8) DMA% : 3.25 (1.06–9.97) InAs% : 3.50 (0.73–16.9)
Chen, 2003	Case Control	Skin Cancer	As3: 2.39(1.02–5.6) Low SMI and high CAE: 7.48(1.65–33.9)

CHAPTER 6: DISCUSSION AND IMPLICATIONS

Summary of the key findings from the Arsenic study

Summarize the three papers here in brief

The three different studies on the inter-relationships between

Implications for the health behavioural model

Andresen and Newhouse (1968) proposed a model on the utilization of and access to health care services for individuals and families. Using national survery data, they identified three main determinants of health care access and utilization they termed as predisposing variables, enabling variables, and need variables. Predisposng variables referred to those variables that were associated with demographic characteristics, the enabling variables were all variables that would allow an individual to access health services such as regular source of income and travel arrangements (such as availabilit of public transport), and need variables that were either physiological/pathological or evaluated need that was determined by a physician or an

The initial model proposed by Andersen underwent four phases of revisions (andersen revbehav paper). The final model is presented in the above figure (Figure 1). Thus, for example, in the initial model, there was no mention of genetic or genome based variables that might account for health services utilization (True and Romeis reference). This variable was added to the model (predisposing category). In addition, the typology was expanded to further classify different forms of access to care (potential versus realized access), and need (realized versus evaluated needs).

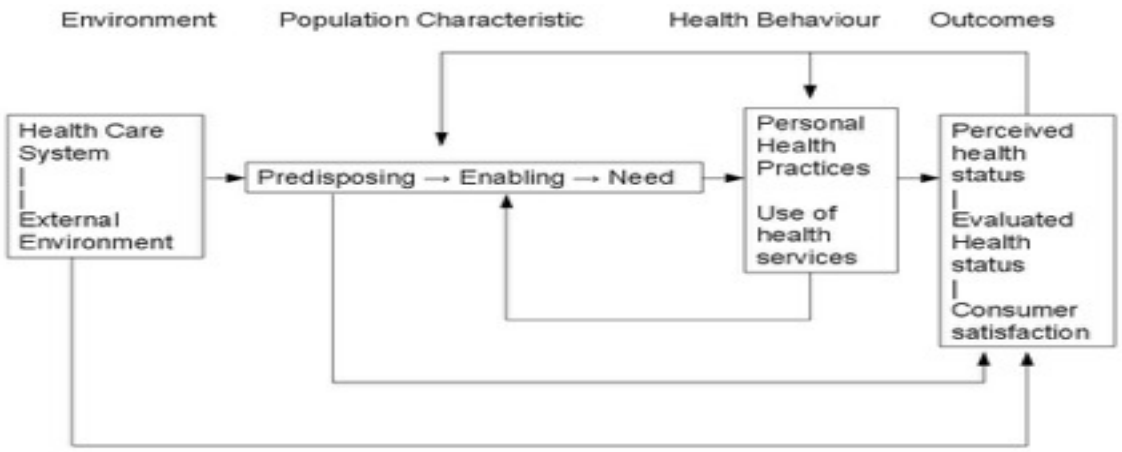

Figure 5: Diagram of the Anderson model of behavioural theory of health care utilization

Figure 5 illustrates the behavioural theory of health services utilization. Andersen and Newhouse (1968) proposed an explanatory and a predictive model of health services utilizaiton for individuals, families, and population. According to this model, health services utilization as an outcome can be both explained and predicted by considering a range of variables. This model has undergone several iteration over three decades to take into account dominant paradigms of health services utilization (cite the 1995 paper).

Central to the Andersen (1995) model is a typology of variables that may explain health care utilization and access based on their analysis of large administrative databases. The typology was based on three mutually exclusive categories pertaining to individual/population characteristics, organization or systems characteristics, or outcomes and named as predisposing, enabling, and need variables.

Predisposing variables are defined as person/population-specific variables that have the property to

affect an individual's propensity to use or access a specific type of service or make one prone (or not) to develop a disease or health state that would lead to uitlization of and access to health service. An illustrative example in the context of mental health is utilization of or access to dementia related service. In a model that would attempt to explain or predict what individuals/organizational characteristics would be more likely than others to be associated with increased utilization of dementia related service, an individual's age would be considered as a predisposing variable. This is because “dementia” by definition includes age dependent slowing down of mental processes, and therefore other than conditions such as progeria or Huntington's disease, older individuals are more likely than younger individuals to develop dementia. Therefore, it is reasonable to speculate that age is predisposing variable in geriatric and mental health and dementia related service utilization and access. As another example, in the context of skin lesions and utlization of dermatological services, one can occupation that involves handling chemicals may be perceived as predisposing variables.

Enabling variables are organizational variables. Enabling conditions make utilization/access to care or utilization of health services possible (or difficult to access conversely) for individuals to access or utlize care. Continuing with the example of dementia, an enabling variable that would be associated with increased access might be availability of trained geriatricians, nurses, gerontologists, behavioral scientists, as well as nursing homes and residential care facilities that are capable of taking care of elderly individuals in need of dementia care that the aged person can easily access. Using the example of skin lesions and dermatological services utilization, an enabling variable might be availability of dermatological expertise and specialist services that the patient can both afford and access.

Need variables indicate the driving force or demand that an individual utlize the available service. For example, in case of dementia, a trigger such as agitation or another form of psychosocial

distress may be considered as a need variable that explains why utilization has occurred for an individual (??). In case of skin conditions, specific lesions such as keratosis that interferes with day to day work or painful lesions or ulcers developing in areas of skin lesions may drive the need to access specialist services related to skin care (??). Andersen identified need variables as prime movers fo health services utilization (??).

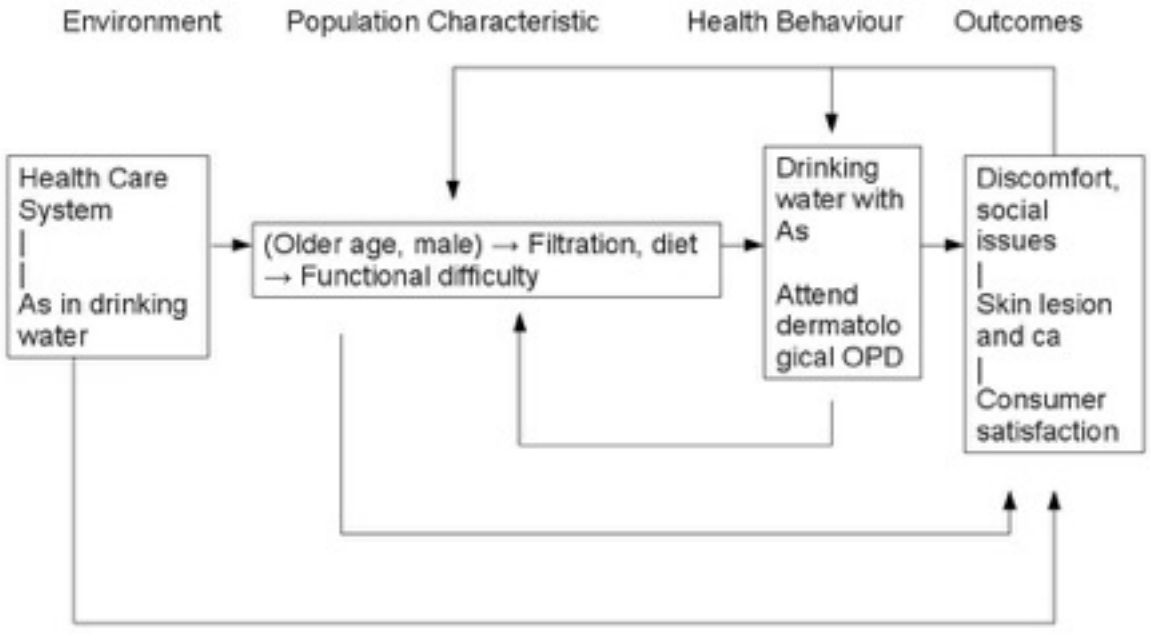

Figure 6: Application of results of Arsenic toxicity studies to Andersen model

Implications for the Halfon and Hochstein model

The life course health development model is based on two fundamental principles – inherent dynamism of health as a state of being and one a state of continual development (the developmental perspective is brought from developmental sciences so that life is being considered as a

continuously developing unfolding process), and the role of adaptability of an individual with changing environment as an individual develops in course of life (hence the term life course health development). The first principle is based on the premise that health is beyond a “state of physical, mental, and social well being”, and is a dynamic resource for everyday living (who current definition) and therefore, according to this model, organization of health services should be aligned to reflect the patter of service utilization by individuals throughout their life span. According to this theory, for individuals in general, life begins at conception in the form of a fertilized egg and proceeds through the stages of embryo, fetus, neonate, infancy, early childhood, preschool years, school-age, adolescence (early, intermediate, and late), early and middle adulthood, late adulthood, leaving the workforce, and older age. Health at any of these stages is a complex interplay of environment, genes, and other societal and life-span factors and these must be taken into account while planning or explaining health service utilization.

The core concepts of ages, and stages of life development are defined in terms of life trajectories, inteaction between micropathways, and macropathways, and adaptability of individuals with their environment. The concept of trajectories in the context of Arsenic toxicity is explained in Figure 3. How life trajectories evolve and develop in course of a person's exposure ot inorganic Arsenic in drinking water supply

can be illustrated by considering a diagram (Figure 1). If functional state of health be plotted along the Y axis and the different stages of life over time be plotted along the X (horizontal) axis, it turns out that at least conceptually, for most persons, functional states are low in the early years of life; it peaks in the most productive years of life in middle and late adulthood, and then drops from the peak position to declining states of functionality as a person ages and enters senescence (refs). However, in reality, for most people, the trajectories are far more complex as there are numerous dimensions (not just functional capacity but there are other measurable outcomes and health states

as well), and each of these states is sensitive to key events in life. According to Halfon et al, events that occur during the developmental stages of an individual can influence late life health events and states. Essentially these events can occur in periods when organs are developing and changes are irreversible (termed as critical periods, such as the first trimester of pregnancy), or there are windows of period during which events can impact the future course of health states of individuals if occur during the embryonic period (termed as : sensitive periods). There are several illustrous examples where events during the sensitive or critical periods have resulted in significant health state alterations that can be traced back to maternal health states. One example is that of the historical example of phocomelia (malformed limbs), other examples include vaginal adenocarcinoma in adolescent girls whose mothers had taken diethyl stilebesterol, malnutrition during pregnancy or low states of early nutrition during fetal stage predisposing a child to develop later diabetes (refs). Thus, dyanmic modification of life trajectories and their resultant effects can explain how and why individuals and populations can utilize specific health services.

The second principle that underlies life span model of healthcare relates to the interplay between macro and micropathways that according to Halfon et al reflects the biological imperative of health services research. Micropathways are the individual biochemical and pathological pathways that account for disease causation and mechanisms. For example, exposure to inorganic Arsenic in drinking water for a prolonged period of time (exceeding six months) and in increased concentrations (about 10 micrograms/L of inorganic As in drinking water) can result in a process where inorganic As is in excess in organs like liver where they are metabolized to monomethyl arsonous acids and dimethyl arsinic acids and thus are soluble in urine and are excreted in kidney. This process involves a step that involves transfer of methyl groups from different sources. However, transfer of methyl group and diversion of methyl groups for the purpose of methylation of inorganic Arsenic can lead in turn to abnormal methylation patterns of other more life sustaining

methylation events such as altered methylation of DNA molecules and lead to abnormal cell proliferation and development of cancer. This in turn is manifest in an early form as Arsenic caused skin lesions (Figure 5).

In the context of environmental health, the underlying micropathway may not always be clear. Enviromental epidemiological studies typically unravel the roles and connections between micropathways and macropathways in the context of environmental health studies. The case of inorganic Arsenic toxicity can be illustrated using macropathways. One possible macropathway that can reverse the process of micropathway and causation of skin lesions and cancer might be limiting or controlling access of the individual to the Arsenic contaminated water supply (??).

[Write something about adaptation].

Life course health development have formed the basis of development of measurable outcomes and have been applied ot develop health services programmes, but they have not been incorporated in explanatory or predictive model building for understanding variability of health service utilizaiton or access (??? write references related to this from Halfon). Therefore, if the elements of life course health development can be combined with a well specified model/framework such as Andersen's health behavioural model, the resulting model may provide a comprehensive framework to explain healthcare utilization and patterns of usage for not only chronic diseases, health states, but also enable modelling of the dynamic nature of shifting health states, health care utilization patterns, and used in conjunction with environmental epidemiological study findings, can provide a robust framework of explaining how physical, built, chemical and biological environment can impact and explain patterns of health service usage and delivery.

In this paper, therefore, we propose a novel framework to explain a wide range of issues around health care access and utilization. We provide a description of the model, and use results from empirical studies to explain and illustrate different components of the model. In this paper, a formal model validation is not reported. The framework is essentially built from two existing frameworks, and thus, hopefully, retains the robustness of both models.

In this paper, we argue that health or disease is a dynamic state where developmental and genetically determined health states interact with with

environmental triggers, and individuals continally adapt and develop throughout their life span (diane kuh + halfon references). Therefore, at any given point in time, healthe services utilization can not only be determined by the personal charateristics (demographic, disease pathological, and healthcare needs), or organizational constucts (variables that enable or deter) an individual's access to or utilization of health care, but there are other complex interplays where environmental events interact with individual biological systems and health outcomes are determined in complex ways that need careful analysis and unraveling using principles of environmental epidemiological studies. This “crossing point” between environment, health, biological imperatives and health services can be illustrated by considering a specific example of Arsenic toxicity and how individuals who live in Arsenic endemic areas and develop various different health effects may access or utilize available health care services. Inorganic Arsenic is present in the shallow aquifer layer of the groundwater in different countries in the world (Argentina, Chile, Southwestern United States, Mexico, several states of Europe, Taiwan, China, Ganges delta (West Bengal state of India and Bangladesh), Mekon Delta and several other areas of the world. Inorganic Arsenic (InAs), once it enters the body through drinking water (or other source – the second most common source of As in the environment is copper smelting

operations), undergoes metabolism in liver and gets converted to methylated arsenicals that are excreted thorugh urine. When individuals are exposed to high concentrations of inorganic As in the environment, the high load of InAs leads to deranged methylation of DNA in the cellular nuclei and in addition, through other mechanisms that are not clear, leads to development of typical skin lesions (melanosis, keratosis, other types of pigmentation, skin cancers, and cancerous lesions in other parts of the body). Other than symptomatic management, there is no known effective pharmacological treatment for arsenic caused skin lesions, and treatment of As caused cancers is same as treatment of organ-specific cancerous lesions. The only prevention of As caused toxicity is treatment of water to remove As from it, or preventing susceptible individuals to access As rich water as drinking source; neither preventive measures are feasible for large population groups that are commonly affected. Given these complexities of environmental events , if Andersen's model of health services utilization and access to care were to be applied to susceptible population in either predicting or explaining access to care and utilization of health services (in this case cancer related services, or dermatological services) in As endemic areas, conceptually, such a model would have to include environmental variables in addition to individual or population specific predisposing and need variables that might account for population in general to access or

utilize available healthcare services for dermatological conditions or skin cancers, or other cancers because the demographic profile, and the needs might be different from that in the other services. Further, the role of enabling variables would necessarily be debatable because As toxicity is essentially anthropogenic, in other words, the response of health system is in response to organizational issues associated with Arsenic in groundwater as a result of human activities. Health effects of As (cancerous effects) may not manifest until years of continuous exposure. Further, in case of arsenic toxicity, empirical studies support (published reports and unpublished analyzed data from this dissertation project) suggest that intake of protein rich diet, calcium and phosphorus may be associated with less likelihood of Arsenic caused skin lesions (cite soma + craig + studies from Bangladesh +). Finally, not all individuals would be equally access health care services even if they were made available depending on their biological propensity to methylate Arsenic. The capacity to methylate Arsenic and therefore protective effects or risk factors of Arsenic toxicity can also be modified by dietary and micronutrient supplementation (mention Gamble's data and unpublished results from this dissertation work). These indicate a need for extending the concepts of Andersen model and incorporate principles of life course health development issues in explaining how individuals access and utilize

health services throughout their life span and how that variability can be incorporated.

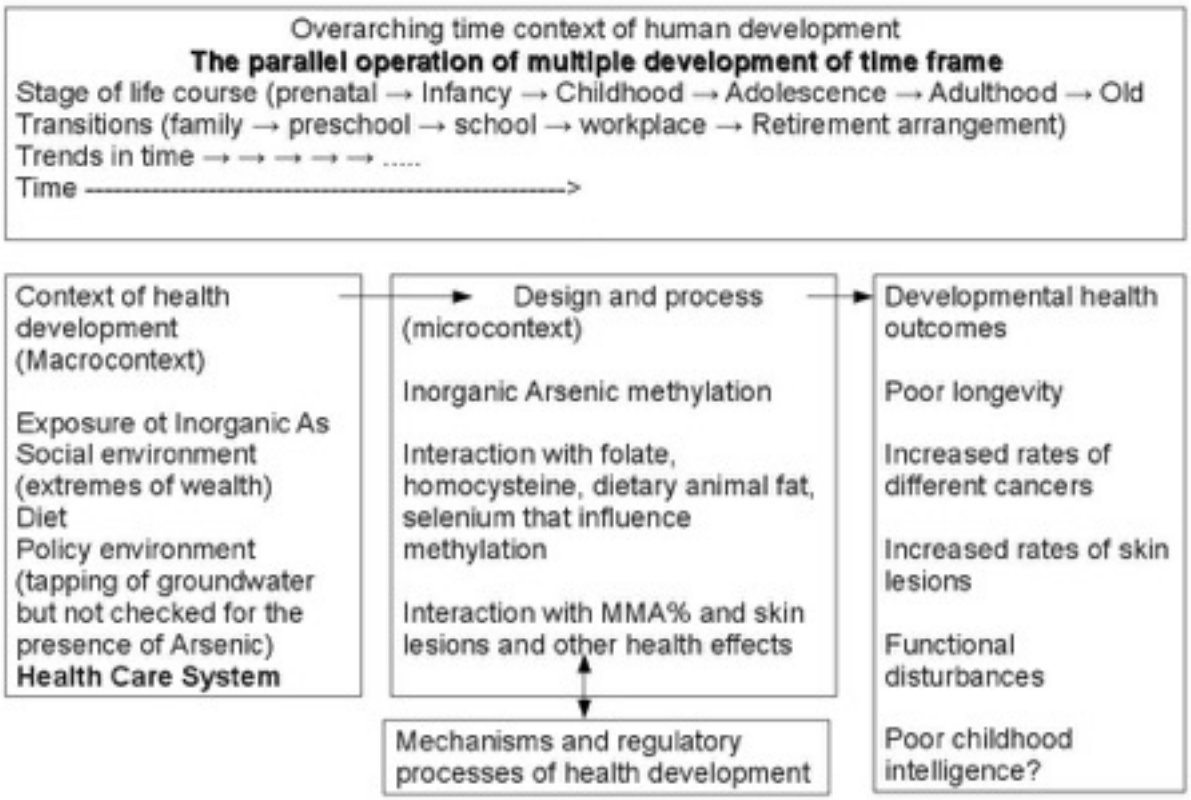

Figure 7: Diagram of the Halfon and Hochstein's model of life course health development

Agenda for future research

APPENDIX

BIBLIOGRAPHY

VIA AUCTORIS