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# A study of telomere length, arsenic exposure, and arsenic toxicity in a Bangladeshi cohort

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ABSTRACT

*Background:* Chronic arsenic exposure is associated with increased risk for arsenical skin lesions, cancer, and other adverse health outcomes. One potential mechanism of arsenic toxicity is telomere dysfunction. However, prior epidemiological studies of arsenic exposure, telomere length (TL), and skin lesion are small and cross-sectional. We investigated the associations between arsenic exposure and TL and between baseline TL and incident skin lesion risk among individuals participating in the Health Effects of Arsenic Longitudinal Study in Bangladesh (2000–2009).

*Methods:* Quantitative PCR was used to measure the average TL of peripheral blood DNA collected at baseline. The association between baseline arsenic exposure (well water and urine) and TL was estimated in a randomly-selected subcohort (n = 1469). A nested case-control study (466 cases and 464 age- and sex-matched controls) was used to estimate the association between baseline TL and incident skin lesion risk (diagnosed < 8 years after baseline).

*Results:* No association was observed between arsenic exposure (water or urine) and TL. Among incident skin lesion cases and matched controls, we observed higher skin lesion risk among individuals with shorter TL ($P_{trend} = 1.5 \times 10^{-5}$) with odds ratios of 2.60, 1.59, and 1.10 for the first (shortest), second, and third TL quartiles compared to the fourth (longest).

*Conclusions:* Arsenic exposure was not associated with TL among Bangladeshi adults, suggesting that leukocyte TL may not reflect a primary mode of action for arsenic's toxicity. However, short TL was associated with increased skin lesion risk, and may be a biomarker of arsenic susceptibility modifying arsenic's effect on skin lesion risk.

## 1. Introduction

More than 100 million people worldwide experience chronic arsenic exposure through naturally contaminated drinking water (World Health Organization, 2001), including approximately 20–45 million in Bangladesh (Flanagan et al., 2012). Arsenic exposure has been reported to increase the risk of various adverse health outcomes including mortality (Sohel et al., 2009), neurological conditions (Vahidnia et al., 2007), cardiovascular diseases (States et al., 2009), as well as malignancies such as cancers of the skin, bladder, kidney, liver, and lung (Celik et al., 2008; Hopenhayn-Rich et al., 1998; Liu and Waalkes, 2008; Mink et al., 2008; Yu et al., 2006). An early and common sign of arsenic toxicity is

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the appearance of skin lesions, which can reflect susceptibility to arsenic-related diseases, including cancers (Yu et al., 2006; Cuzick et al., 1982, 1992; Hsu et al., 2013; Arsenic in Drinking Water, 2001).

The mechanism of arsenic's toxicity and carcinogenicity may be related in part to telomere length and dysfunction. Telomeres are the repeating six-nucleotide sequence binding protein complexes at the end of human chromosomes that protect DNA ends from damage. The telomerase enzyme elongates telomeres in stem and progenitor cells (Shawi and Autexier, 2008). Telomere length (TL) shortening occurs with cell division and has been investigated as a biomarker of aging and susceptibility for age-related health conditions, including cardiovascular diseases, neurocognitive diseases, cancers, and overall mortality (Cawthon et al., 2003; Martin-Ruiz et al., 2006; Sanders and Newman, 2013; Serrano and Andrés, 2004; Willeit et al., 2010), all of which have also been linked with chronic arsenic exposure. *In vitro* studies of human cord blood cells and human cell lines have shown that arsenic exposure can both increase and decrease TL and telomerase activity depending on dose (Ferrario et al., 2009; Zhang et al., 2003). Acceleration of TL shortening has been attributed in part to oxidative stress and inflammation (Jenny, 2012; O'Donovan et al., 2011; Von Zglinicki, 2002), two processes that are also potential causes of arsenic toxicity (Ahmed et al., 2011; Kitchin, 2001; Liu et al., 2003).

Recent epidemiologic studies linked arsenic exposure to longer TL in peripheral blood and saliva (Chatterjee et al., 2015; Fillman et al., 2016; Gao et al., 2015; Li et al., 2012; Ameer et al., 2016) and altered peripheral blood expression of genes involved in telomere maintenance (Gao et al., 2015; Li et al., 2012; Mo et al., 2009). However, the few published studies of the association between arsenic exposure and TL tend to be small, and only one study to date has investigated the association between TL and arsenical skin lesions—a cross-sectional study that found longer TL in skin lesion subjects (Chatterjee et al., 2015). The goal of this paper was to characterize the role of TL in the arsenic-skin lesion association by answering two questions: 1) whether arsenic exposure is associated with TL; and 2) whether TL is associated with skin lesion risk. Addressing these questions will allow us to determine whether TL is a mediator or modifier of the association between arsenic and skin lesion. In animal models, shorter telomere length has been associated with skin lesions including ulcerations with epidermal hyperplasia and hyperkeratosis (Rudolph et al., 1999; Varela et al., 2016). UV radiation-induced short TL has previously been linked to actinic keratosis in human subjects, suggesting that TL may play a role in the biology of skin lesions independent of any arsenic exposure (Ikeda et al., 2014). Furthermore, given that age is a risk factor for skin lesion (Ahsan et al., 2006), and TL is related to multiple diseases of aging (Haycock et al., 2017), we additionally hypothesize that TL may independently affect skin lesion risk. Prospective studies assessing the association between baseline TL and risk of subsequent arsenic-related skin lesions are needed to establish the temporal relationship between TL and skin lesion risk, allowing for the evaluation of TL as a biomarker for susceptibility to arsenic-related skin lesions in exposed populations.

In this study, we assessed the associations between arsenic exposure and TL and between baseline TL and subsequent skin lesions among Bangladeshi individuals with a wide range of arsenic exposure through drinking water.

## 2. Methods

### 2.1. Study participants

The Health Effects of Arsenic Longitudinal Study (HEALS) in Araihazar, Bangladesh is a prospective cohort study designed to assess the effects of exposure to arsenic through drinking water on health. Details of the participant selection, study design, and study methods have been described previously (Ahsan et al., 2005). In brief, 11,746 male and female participants were recruited between 2000 and 2002 in Araihazar, Bangladesh. The study location is in a rural area with relatively homogeneous sociocultural characteristics. The cohort has been expanded in subsequent years but the current study is conducted among this original cohort. Demographic and lifestyle data as well as blood (from which TL is measured) and urine samples were collected at baseline. In-person visits were conducted every two years following the baseline visit and each visit included a physical examination and collection of urine. All subjects in the study received basic medical care through the study clinic. Informed consent was obtained from all study participants, and the study protocol was approved by the Institutional Review Boards of the University of Chicago, Columbia University, and the ethical committee of the Bangladesh Medical Research Council.

### 2.2. Skin lesion status

During the baseline interview as well as three biennial follow-ups, participants were examined by trained physicians for the presence of arsenical skin lesions according to a structured protocol. The physicians, who were blinded to the participants' arsenic exposure status, recorded the presence of melanosis (hyperpigmentation), leucomelanosis (hypopigmentation), or keratosis (thickening of the skin on palms and soles). For this study, skin lesion status was defined as the presence or absence of any type of the aforementioned skin lesions. Over the course of the three biennial follow-ups after baseline, 866 individuals developed incident skin lesions among the 10,182 skin lesion-free individuals at baseline (Argos et al., 2011).

### 2.3. Selection of the subcohort

A random subset of the baseline HEALS cohort was selected (n = 1469), including participants with prevalent (i.e. pre-existing) skin lesions at baseline (Ahsan et al., 2006; Argos et al., 2011). Although we have arsenic exposure measurements for a larger sample of subjects from the Bangladesh Vitamin E and Selenium Trial (BEST) (n = 1825) and the expansion of the HEALS cohort (ACE) (n = 1047), only HEALS subjects were included in the analysis in order to avoid any issues of exposure misclassification. HEALS subjects' baseline exposure reflects historical exposure as participants were not aware of the arsenic exposure status at baseline (Chen et al., 2007), whereas many BEST and ACE participants may have been aware of their exposure status at baseline and have had the opportunity to change their exposure in response to arsenic mitigation efforts. We chose to conduct this analysis in a randomly-selected subcohort rather than in a selected group of skin-lesion cases and/or controls in order to avoid collider bias (Cole et al., 2010). Collider bias is a form of selection bias in which a spurious association is induced between two variables when the analysis is conditioned on a common outcome (e.g. conditioning on skin lesion status, a potential common effect of both arsenic exposure and TL). TL measures were not obtained for the full cohort due to limited resources.

### 2.4. Nested case-control study selection

To assess associations between TL and skin lesion status, we conducted a nested case-control study of 516 individuals with incident skin lesions who were frequency matched by sex and 5-year age intervals with 516 individuals who remained skin lesion-free during the study. Subjects selected for the nested case-control study partially overlap with the random subcohort, but also include additional individuals from the parent HEALS study. We chose to perform age- and sex-matching for this analysis because age and sex are well-described correlates of TL (Sanders and Newman, 2013), which may confound the association between TL and skin lesion status. Among the subjects selected for the nested case-control study, samples for 92 subjects were excluded from the analysis due to TL assay quality control measures as detailed below, resulting in 466 cases and 464 controls. Fig. 1 shows a flowchart of the selection of participants for the nested case-control study. Characteristics of subjects excluded due to insufficient DNA and