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Association of cadmium and arsenic exposure with salivary telomere length in adolescents in Terai, Nepal

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ABSTRACT

Background: Cadmium and arsenic are ubiquitous metals commonly found in the environment which can harm human health. A growing body of research shows telomere length as a potential biomarker of future disease risk. Few studies have examined the effects of metals on telomere length and none have focused on adolescents.

Objectives: In this study, the impact of cadmium and arsenic on salivary telomere length was studied in adolescents in Terai, Nepal.

Methods: Adolescents aged 12–16 years old ($n=351$)were recruited where questionnaire interviews and both saliva and urine collection took place. Telomere length was determined by quantitative polymerase chain reaction using DNA extracted from saliva. Urinary cadmium and arsenic concentration were measured by inductively coupled plasma mass spectrometry. Multivariable linear regression was used to examine associations between urinary metals and salivary telomere length.

Results: The geometric means and standard deviations of cadmium and arsenic were 0.33 ± 0.33 μg/g creatinine and 196.0 ± 301.1 μg/g creatinine, respectively. Urinary cadmium concentration was negatively associated with salivary telomere length after adjustment for confounders ($\beta=-0.24$, 95% CI $-0.42, -0.07$). Arsenic showed positive associations with telomere length but did not reach statistical significance.

Conclusions: This is the first study to demonstrate that cadmium may shorten adolescent telomeres, even at exposure levels that may be considered low. These results agree with prior experimental and adult epidemiological studies, and also help identify the mechanism of DNA damage by cadmium. This study expanded current evidence on the harmful effects of cadmium exposure on telomere length even to adolescents.

1. Introduction

Exposure to metals such as arsenic and cadmium has been recognized as an urgent public health issue, especially in many developing countries. Both metals can have harmful effects on health, for arsenic including skin manifestations, cardiovascular disease, and cancer of many organs (IPCS, 2001) and for cadmium including detrimental effects on the kidney and bones, cardiovascular disease, and cancer (ATSDR, 2012; IPCS, 1992). Exposure to arsenic can occur from drinking water and plant consumption (IPCS, 2001) and exposure to cadmium can also occur from plant consumption (ATSDR, 2012), particularly rice in Asian countries (Rivai et al., 1990; Tsukahara et al., 2003), or cigarette smoking (IPCS,1992).

Recent evidence has suggested metal toxicity has deleterious effects on telomeres. Telomeres are repeat nucleotide sequence "caps" located at the ends of chromosomes that function to protect the genetic data of chromosomes. Telomeres get shorter with every cell replication until the end replication problem occurs, where during cell division DNA polymerase cannot completely replicate the end telomeric DNA (Blackburn, 2005). Thus, telomere length

Abbreviations: BMI, body mass index; PCR, polymerase chain reaction; T/S ratio, telomere/single copy gene ratio; ICP-MS, inductively coupled plasma mass spectrometry; LOD, limit of detection

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has been proposed as a marker of biological aging, or cumulative organismal aging, and suggested as a marker of future disease risk. Telomeric DNA is particularly sensitive to oxidative stress (Von Zglinicki, 2002) and oxidative stress is also a pathway for metal toxicity (Valko et al., 2006). The enzyme telomerase can add nucleotide sequences to the ends of telomeres but is present at low levels in most tissues. Shorter telomeres and senescent cells have been observed in degenerative tissues (Blasco, 2005). Experimental evidence has shown that cadmium in embryonic stem cells resulted in oxidative stress, DNA damage, and telomere shortening at a lower concentration and apoptosis and DNA damage at an acute high concentration (Huang et al., 2013, 2010). Arsenic may have differing effects on human cell telomeres depending on the concentration: telomere length maintenance and increased telomerase expression at a lower concentration, versus shortening of telomeres, apoptosis, increased reactive oxygen species and decreased telomerase expression at a higher concentration (Ferrario et al., 2009; Zhang et al., 2003). These mechanisms may be involved in the carcinogenic effects of each metal.

Telomere attrition has been associated with a number of age-related diseases and health outcomes in adults, including inflammation (Wolkowitz et al., 2011), cardiovascular disease (Fitzpatrick et al., 2007), type II diabetes (Salpea et al., 2010; Sampson et al., 2006), cancer (McGrath et al., 2007; Willeit et al., 2010), dementia (Martin-Ruiz et al., 2006; Von Zglinicki et al., 2000), mortality (Cawthon et al., 2003; Kimura et al., 2008), as well as psychosocial factors (Epel et al., 2004). Several studies have also established links between change in telomere length over time and health in children (García-Calzón et al., 2014; Shalev et al., 2013) as well as between shorter telomere length in adults and childhood diseases (Entringer et al., 2011; Li et al., 2014; O'Donovan et al., 2011; Shalev et al., 2014; Tyrka et al., 2010). The relationship between childhood telomere length and later life disease remains unclear.

Epidemiological research has also started to examine the impact of heavy metal exposure on telomere length. Only one study has been carried out on children, examining 8 year olds in Poland near an industrial pollution site, which did not show significant effects of blood cadmium on telomere length (Pawlas et al., 2015). Adult studies in China, measuring placental telomere length and cadmium of pregnant women living near an electronic waste site, and in the U.S., examining blood telomere length and urinary cadmium in a nationally representative sample from NHANES 1999–2002, have shown shorter telomere length with higher cadmium exposure (Lin et al., 2013; Zota et al., 2015). Adult studies in Bangladesh, India, and Argentina have also examined urinary arsenic exposure and blood telomere length, finding longer telomeres with increased arsenic exposure (Chatterjee et al., 2014; Gao et al., 2015; Li et al., 2012). No studies have focused on adolescents, despite that adolescence is a critical window of child growth and development and thus susceptible to chemical insults (Landrigan et al., 2004), particularly in endocrine, reproductive, and neurological areas (Carpenter and Bushkin-Bedient, 2013).

Previous research has shown that 25.7% of the tube wells in the Nawalparasi District of Terai, Nepal are contaminated with arsenic above the 50 μg/L limit for Nepal (Maharjan et al., 2005). In addition, despite the potential for cadmium exposure due to the high rice consumption in this area (Parajuli et al., 2012), cadmium exposure has not been investigated. In the present study, we examined the effects of urinary cadmium and arsenic exposure on salivary telomere length in adolescents in Terai, Nepal.

2. Methods

2.1. Study area

This study was carried out in Terai, Nepal, in the district of Nawalparasi on the central southern border of Nepal. The Terai flatlands are located south of the Himalayas with low altitudes ranging from 60 to 310 m above sea level. The study area was in a very rural area composed of mainly agricultural fields of paddy and wheat, where parents of study participants were primarily farmers or laborers. This district was chosen based on previous studies showing the presence of arsenic-contaminated tube wells (Maharjan et al., 2005), as well as a diet with high rice intake (Parajuli et al., 2012).

2.2. Study participants

Participants were 12–16 year old adolescents with no known medical conditions. Participants were recruited from January to April 2014 at two secondary schools in the highly affected arsenic area of Nawalparasi district, Nepal. Participants with both consent and assent whom were in the eligible age range of 12–16 years as confirmed by birth certificate birth date were invited for participation. The response rate was 92% and of the 372 eligible participants, 21 were excluded due to missing demographic or sample information, leaving a total of 351 adolescents who were included in this study.

2.3. Anthropological data and biological sample collection

Anthropological data was collected for weight and height, and used to calculate BMI as weight (kg) divided by height (m) squared. Two mL passive drool saliva samples and 4 mL urine samples were collected by trained local health workers in polyethylene cryovials and frozen after collection. Passive drool saliva samples were collected after confirmation that consumption of food, alcohol, smoking, or vigorous exercise did not occur in the last hour. Participants were asked to allow saliva to passively pool in the mouth and into the collection tube. Frozen samples were transported to The University of Tokyo on dry ice and stored at −80 °C until further analysis.

2.4. Questionnaire survey

Local health workers were trained to administer questionnaires. Socio-demographic information of adolescents was collected on age, school grade, address, maternal age at birth, and parental education level. Participants were asked about their medical history and current medications and those with abnormalities were excluded from the study.

2.5. Measurement of telomere length from salivary DNA

Genomic DNA was extracted from 1 mL of passive drool saliva treated with PBS (−) into a final 200 μL solution using the isolation of genomic DNA from saliva spin procedure provided in the QIAamp DNA Blood Mini Kit (Qiagen K. K., Tokyo, Japan) based on silica-gel DNA binding membrane spin columns. DNA was eluted into a 100 μL buffer following the manufacturer's protocol. Telomere length was examined using DNA extracted from saliva by a real time quantitative polymerase chain reaction (PCR) method developed by Cawthon (Cawthon, 2002) with some modifications (Lin et al., 2010). In brief, the quantitative PCR method determines a T/S ratio, the ratio of telomere repeat copy number (T) to single copy gene number (S) for each sample in comparison to a reference DNA sample. PCR reactions, 20 μL in each, were carried out using the Roche Light Cycler Essential