Using hair and fingernails in binary logistic regression for bio-monitoring of heavy metals/metalloid in groundwater in intensively agricultural areas, Thailand

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\begin{abstract}
In this study, the hair and fingernails of the local people in an intensively cultivated agricultural area in Ubon Ratchathani province, Thailand, were used as biomarkers of exposure to arsenic (As) and heavy metals. The study area has shallow acidic groundwater that is contaminated with As and heavy metals. The local people often consume this shallow groundwater; thus, they are exposed to As and heavy metals. Hair and fingernail samples were collected to characterize the differences between shallow groundwater drinking (SGWD) and tap water drinking (TWD) residents. The concentrations of As and the heavy metals Cd, Pb and Hg were significantly higher in the hair samples from the SGWD group than those from the TWD group, especially for As (0.020–0.571 vs. 0.024–0.359 µg/g) and Cd (0.009–0.575 vs. 0.013–0.230 µg/g). Similarly, the concentrations of As and the heavy metals in the fingernail samples collected from the SGWD group were larger than those of the TWD group, especially for As (0.0.039–2.440 µg/g vs. 0.049–0.806 µg/g). The $\chi^2$ statistic and binary logistic regression were used to find the associated factors and assess the associated probabilities. The regression results show that the factors associated with the concentrations of As and the heavy metals in the hair samples were drinking water source, rate of water consumption, gender, bathing water source, education, smoking and underlying disease, whereas the factors associated with the concentrations of these species in the fingernail samples were drinking water source, gender, occupation, work hours per day, alcohol consumption, and the use of pesticides.
\end{abstract}

1. Introduction

The contamination of groundwater supplies with metalloids, such as arsenic (As), and heavy metals is currently a major environmental concern. Many countries currently use groundwater as their main source of fresh water (Tai et al., 2012). Heavy metals and metalloids cause harm to water resources, given that they are strongly toxic even at low concentrations (Marcovecchio et al., 2007). Heavy metals and metalloids enter the human body via three main routes, namely inhalation, transdermal adsorption and ingestion. Their compounds accumulate primarily in bones, internal parenchymal organs, myocardial tissue, skin and hair (Michalak et al., 2012). Adverse health effects associated with chronic ingestion of arsenic in drinking water have a significant effect on public health worldwide (Adair et al., 2006).

This study focuses on local people who have lived permanently (without migrating) for a long period at a site with intensive agriculture in Ubon Ratchathani province in northeastern Thailand. The groundwater in this area has been found to contain substantial amounts of toxic heavy metals and As (Wongsasuluk et al., 2014). It is a farming area with a high density of agricultural sites that have used agrochemicals (fertilizers, herbicides and pesticides) in the cultivation of various agricultural products, such as chilies, rice, and rubber trees, for
more than 30 years (Norkaew et al., 2009). In this area, most of the local people live in homes that are located on farms and have no electricity or tap water. As a result, shallow groundwater is the major source of fresh water for both agricultural activities and human consumption. Shallow groundwater from farm wells at agricultural sites is an important source of drinking water for most of the local people (Chotpantarat et al., 2014; Wongsasuluk et al., 2014). Contamination of this shallow groundwater with heavy metals and As leads to accumulation of these species in the human body, principally via drinking and (to a lesser extent) by bathing. Long-term exposure to these species can cause adverse health effects, even at low concentrations (Siriwong, 2006; Chotpantarat et al., 2011a, 2011b; Wongsasuluk et al., 2014, 2017). Furthermore, the risks to human health associated with the contamination of groundwater by heavy metals and As have been reported from this area, and both non-cancer-related and cancer-related health risks associated with drinking the shallow groundwater over a long period of time have been reported (Chotpantarat et al., 2014; Wongsasuluk et al., 2014).

To support risk assessments for sites with low levels of contamination, the use of biomarkers is used as evidence that heavy metals and As can accumulate in the human body, where they can cause adverse health effects. The hypothesis examined in this study is that the shallow groundwater drinking (SGWD) residents should have higher concentrations of heavy metals (Cd, Pb and Hg) and As in their hair and fingernails than the tap water drinking (TWD) residents in the same agricultural area, despite the low concentrations of As and heavy metals in the groundwater.

2. Materials and methods

2.1. Study area

This study site is located within the largest agricultural area in Ubon Ratchathani province, which makes up a part of northeastern Thailand. This study site is located within a range of UTM northings of 1695000–1704000 and a range of UTM eastings of 479000–469000 (Fig. 1). The area has been continuously and intensively cultivated over a long period, and it contains the largest chili farms in Thailand (Norkaew, 2009). In addition to chilis and rice, this site produces many other agricultural products, such as rubber trees and corn (Wongsasuluk et al., 2014, 2017).

The characteristics of this study site include elevations that range from 87.00 to 147.00 m above mean sea level (amsl). The direction of shallow groundwater flow in this area currently passes from an upstream reservoir and goes down to the southern part of the study area. The groundwater is fairly shallow and mainly settles in a discharge area. Furthermore, the soil textures at this site include three similar types, which are sandy loam, loamy sand and sand (Chotpantarat et al., 2011a, 2011b; Masipan et al., 2016). Moreover, previous studies have shown that the groundwater in this agricultural area is acidic and shallow; the pH is approximately 4 (Wongsasuluk, 2010, 2014). Intensive farming appears to move heavy metals from the soil surface into the shallow groundwater. For more information on the study area, see Wongsasuluk et al. (2017).

2.2. Sampling and analytical methods

The 100 volunteers were local participants living in the study area. The group as a whole included 58 SGWD and 42 TWD participants. The selection of these volunteers followed the inclusion conditions listed below; however, it was otherwise conducted at random from among those who had lived permanently in this agricultural study area without migration or changing their place of residence, were above the age of 18 and had not performed any hair coloring, perming, re-bonding or other chemical activities for at least 1 year prior to the sampling date. In order to be included in the SGWD group, the participants must also have principally drunk water from a shallow groundwater well in study area, whereas inclusion in the TWD group required that the participants drank principally tap or bottled water, instead of shallow groundwater.

All of the selected participants were informed about the hair and fingernail sample collection in face-to-face interviews before the samples were collected. All of the participants consented to the study. Hair and fingernail samples were then collected from each participant, and a questionnaire was used in a face-to-face interview to collect their personal information. The interview questionnaire consisted of two main parts, both of which consisted of open- and closed-ended questions. The first part elicited general information, such as the background and personal information of the participants. The second part focused on the health of the participants and exposure-related information, to enable investigation of the factors that are potentially associated with exposure to As and heavy metals.

The participants were interviewed to fill out a personal information questionnaire, including questions designed to assess hair lifestyle habits (hair coloring, bleaching, straightening or re-bonding). Hair was sampled from the back of the head (around the upper neck) or behind the ear with stainless steel scissors made especially for hair cutting. This study sampled the newly grown hair of participants. Therefore, the first 1–2 cm from the scalp, which represent the last 1–2 months of past exposure, were sampled. The average growth rate of human hair is approximately 1 cm per month and 1 mm per month for nails (Al-Delaimey, 2002; Karlen et al., 2011; Kim et al., 2014; Michalak et al., 2012; Sauve et al., 2007; Wright et al., 2015). Fingernail samples were collected using stainless steel nail clippers. The hair and fingernail samples were sealed separately in labelled polyethylene zip lock bags and were opened only upon reaching the laboratory, when the samples were washed and cleaned (Gault et al., 2008).

The hair samples were cleaned by washing with baby shampoo in the laboratory to remove dust and particles (Kral et al., 2013). Washing the hair affected the sodium content of the hair samples. Later in the washing step, the hair samples were purified by removing organic compounds (Mikulewicz et al., 2011; Michalak et al., 2012). The hair samples were cut into pieces that were 5 mm in length and were washed with acetone (Razi et al., 2012). The sample preparation followed the guidelines of the International Atomic Energy Agency (IAEA), and the samples were washed with acetone to remove external contamination (Li et al., 2011). To prepare the fingernail samples, the visible dirt or surface contaminants were removed by brushing. The samples were then placed in an ultrasonic bath; finally, the samples were washed with acetone. After all of the cleaning procedures had been carried out, the samples were dried in an oven (Samanta et al., 2004).

Following cleaning, 10 mg of each fingernail sample and 100 mg of each hair sample (Gault et al., 2008) were purified of organic matter using 10 ml of 65% (v/v) nitric acid. The Milestone digestion method (Milestone Microwave Laboratory System, 1998) was followed to ensure the complete digestion of the hair and fingernail samples. The concentrations of As and heavy metals in each extracted hair and fingernail sample were determined using Inductively Coupled Plasma Spectrometry-Mass Spectrometry (ICP-MS) (Samanta et al., 2004). Lemos and de Carvalho (2010) reported the LOD (limit of detection) for Cd and Pb in fingernails, which was 0.1 ng/g for Cd and 1.5 ng/g for Pb, using ICP-MS (Batista et al., 2008). Additionally, the determination of Cd and Pb in hair by ICP-MS digestion with the nitric acid method showed that the LOD for Cd was 0.0003 ng/mg and was 0.0003 ng/mg for Pb (Shariati et al., 2008).

Samples of the drinking water (tap water and shallow groundwater) consumed by the local people were collected in rinsed polyethylene bottles. The groundwater was obtained from local wells that the participants generally used as their major sources of drinking water. The samples were acidified with nitric acid to prevent precipitation (Normandin et al., 2014; UnChalee, 2014; Wongsasuluk et al., 2014, 2017). All of the drinking water samples were refrigerated at 4°C in
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