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DETERMINATION OF ARSENIC INTAKE BY DUPLICATE DIET PORTION SAMPLING METHOD AMONG RONPHIBUN RESIDENTS

การตรวจหาปริมาณการได้รับสารหนูโดยวิธี Duplicate Diet Portion Sampling Method ในผู้อาศัยที่ร่อนพิบูลย์

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Abstract

Total daily dietary arsenic intake by adults in Ronphibun district, within Thailand has been re-evaluated via duplicate portion sampling and atomic absorption spectrophotometer determination of the arsenic. Hundred duplicate diet samples were collected from participants. The ranges of arsenic concentration in diets were between 0.49 and 1.33 μg/g, lower than the Thailand Standard value of 2 μg/g. The daily intake of total arsenic in the Ronphibun villagers ranged between 130.72 and 550.26 μg/d with a mean of 274.97 μg/d (SD = 74.78 μg/d).

Daily arsenic intakes of males (mean ± SD; 297.89 ± 87.95 μg/d) were statistically higher than females (mean ± SD; 252.06 ± 51.75 μg/d) (p - value < 0.05).

Keywords: Arsenic, Duplicate Diet, Ronphibun, Atomic Absorption Spectrophotometer

บทคัดย่อ

การประเมินการได้รับสารหนูทั้งหมดจากอาหารที่บริโภคในแต่ละวันของประชากรที่อยู่ในวัยผู้ใหญ่ของอำเภอร่อนพิบูลย์ผ่านการเก็บตัวอย่างแบบ duplicate portion sampling และวิเคราะห์ปริมาณสารหนูด้วย atomic absorption spectrophotometer จากจำนวนตัวอย่างอาหาร 100 ตัวอย่าง พบว่า ปริมาณสารหนูมีค่าระหว่าง 0.49-1.33 ไมโครกรัมต่อกรัม ซึ่งต่ำกว่าค่ามาตรฐานของประเทศไทยที่กำหนดไว้ คือ 2 ไมโครกรัมต่อกรัม การประมาณ

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ค่าปริมาณการได้รับสารหนูต่อวันของประชาชนในร่อนพิบูลย์มีค่าระหว่าง 130.72 - 550.26 ไมโครกรัมต่อวัน และมีค่าเฉลี่ย คือ 274.97 ไมโครกรัมต่อวัน (ค่าเบี่ยงเบนมาตรฐาน = 74.78 ไมโครกรัมต่อวัน) โดยพบว่าเพศชายได้รับสารหนูผ่านการบริโภคอาหารต่อวัน (ค่าเฉลี่ย $\pm$ ค่าเบี่ยงเบนมาตรฐาน; 297.89 $\pm$ 87.95 ไมโครกรัมต่อวัน) ในปริมาณที่สูงกว่าเพศหญิง (ค่าเฉลี่ย $\pm$ ค่าเบี่ยงเบนมาตรฐาน; 252.06 $\pm$ 51.75 μg/d) อย่างมีนัยสำคัญทางสถิติ (p-value < 0.05)

คำสำคัญ: สารหนู, Duplicate Diet, ร่อนพิบูลย์, Atomic Absorption Spectrophotometer

Introduction

The effect of arsenic exposure on human health where observed in populations of South and Southeast Asia; such as, Bangladesh, India, Taiwan, and Thailand. Ingestion of water and food polluted with arsenic has been proven to adversely affect health. Symptoms associated with arsenic toxicity include hyperkeratosis of hands and feet, liver dysfunction, epithelioma, and other cancers of the skin, as well as of the liver and kidney.[1] The first case of arsenical skin cancer in Thailand was reported in 1987 in Ronphibun District of Nakron Si Thammat Province. In the area, this sickness is called **"**Kai-Dam**"** because it has dermatological symptom of creating dark spots on the skin to a hardening of the skin into nodules, often on the palms and soles. During 1987 to 1988, The Ministry of Public Health reported that 1,150 cases identified as having arsenical skin lesions and 818 (85%) patients were recorded as residents in Ronphibun Sub-district. There have four highly arsenic contaminated villages in this sub-district that should be study in details. The reports in 1994 showed that 162 of 616 participants were identified as the patients with arsenical skin manifestations (prevalence rate of 26.3%). By the late 1990s, around 1,500 people have been diagnosed with arsenic related skin.[2, 3] In 2000, the epidemiological survey by Siripitayakunkit[4] showed the prevalence rate of 24.7% by using the skin lesion for selection criteria. Arsenic contamination of the environment by the mining process that occurred in the area for a hundreds year, caused the disease. At present, mining activities which related to arsenic contamination are banned by the Department of Mineral Resources. However, arsenic contamination caused by past mining activities remains in the area. Food crops may accumulate arsenic through root uptake from contaminated soil or water while animals can accumulate arsenic from contaminated feed, sediment and water. Human may be exposed to arsenic from food, water, soil, and air. However, the average daily intake of arsenic was estimated more then 90% coming from water and food consumption.[1, 5]

Three basic approaches for sampling food are used: individual food products; market basket studies; and duplicate diet portion. The duplicate diet approach is a direct sampling technique in which an exact duplicate of food being consumed is obtained and analyzed. Arsenic concentration may differ between cooked and uncooked food. Duplicate diet methods are considered to be more

accurate at estimating personal exposures because they account for individual food and water sources, types and quantities of food items consumed, and cooking methods.[6]

Since data on arsenic intake via duplicate diets of Ronphibun residents are infrequent and dated from long ago.[7] The aim of this study was to actualize the dietary intake of arsenic collected using by duplicate diet portion sampling method in adults living in arsenic-contaminated area, Ronphibun, Thailand.

Materials and Methods

Instruments and Reagents

The quantification of arsenic was performed with an atomic absorption spectrophotometer (AAS) model 300 Perkin-Elmer equipped with an autosampler AS90 and flow injection system (FI-HG-AAS). Standard Reference Materials 1573a (tomato leaves) was obtained from the National Institute of Standards and Technology (NIST), USA. Deionized water (18 MΩ cm), obtained with a Milli-Q water system was used for preparation of standards and reagents throughout the study. All chemicals were analytical grades and purchased from Merck®. All glassware was treated with 10% (v/v) HNO_3 for 20-24 h and washed three times with deionized water before it was used.

Sample Collection

The present study was focused on the 4 villages of Ronphibun Sub-district include villages number 1, 2, 12, and 13. Because almost (>85%) all the patients that suffered from arsenic chronic have lived in these areas.[2] The data from local provincial office reported that total 11,005 people have been living in this sub-district and 2,289 people in village number 1, 2, 12 and 13. Hundred duplicate diet samples were collected from participants (50 females and 50 males; aged: 22-56 years; occupations: 90% farmer and 10% other occupations) during April 2008 to January 2009. The samples were com pleted and of normal composition as provided to a healthy population. The duplicate samples were distributed to all volunteers participated in this projects The participants were briefed at the beginning and throughout the study. The briefing included detailed information on the goal and the background of the study with instructions on how best to collect the duplicate portion of the diet. Each diet sample was collected in separate plastic bag. Drinking water and beverages samples were collected from the present drinking water sources of each participant in clean bottles. After the collection phase, the samples were sent daily to the laboratory in Bangkok.

Sample Preparation

In the laboratory, inedible parts of the foods (e.g. bone, seed of fruits) were discarded. Edible parts were minced and mixed in all duplicate diets and was blended to give a homogeneous sample. The sample was weighed, frozen, freeze-dried, and stored in polyethylene bags at 4 °C until analysis. In this study, beverage intake

rate was not reported because it was embedded in the expression used for amount of arsenic concentration in duplicate diet sample (μg/g, dry weight). Based on the method described by Ruangwises N. and Ruangwises S.[8], an accurately weighed (0.5 ± 0.01 g) lyophilized samples were digested overnight in 15 ml of a mixture of concentrated HNO_3:$HClO_4$ (10:1). The digests were gently evaporated to dryness on a hot plate at 80 °C and then were ignited at 550 °C in a furnace for 3 h. The residues were dissolved in 5% of HNO_3. The resulting solution was used for determination of arsenic by FI-HG-AAS. The accuracy and precision of the analyses for arsenic was checked against the Standard Reference Materials 1573a (tomato leaves).

Statistical analysis

Descriptive statistics were calculated, including mean ± SD. A Student's *t*-test was used to test for significant differences between the arsenic concentrations in different genders. Statistical significance was considered when p-value < 0.05.

Results and Discussion

Arsenic Analysis

Quality control data for analysis of arsenic in samples were quite good. Arsenic concentrations determined in the SRMs 1573a (tomato leaves) was 0.103 ± 0.017 μg/g, which agreed well with the certified levels of 0.110 ± 0.027 μg/g.

Arsenic in Duplicate Diet

Water and food is generally the principal contributor to the daily intake of arsenic. Various sampling methods may be employed in order to obtain the dietary intake of trace elements. Study designs that include collection and preparation of foods ready for consumption are supposed to produce the most realistic and reliable results.[6] In this study, we have used duplicate portion analysis.

Arsenic concentrations found in all the samples analyzed were significantly lower than the maximum permissible concentration limit for arsenic in foods reported by Ministry of Public Health, Thailand, 2 μg/g.[9] In the present study, arsenic concentrations in duplicate diets were in ranges 0.49-1.33 μg/g (mean ± SD; 0.78 ± 0.16 μg/g). The ranges of daily arsenic ingestion rates were 130.72-550.26 μg/d, (mean ± SD; 274.97 ± 74.78 μg/d) and arsenic intakes were 2.56-9.31 μg/kg body weight/d (mean ± SD; 4.71 ± 1.42 μg/kg body weight/d) (Table 1). Body weight and weight of food intake were 42-89 kg (mean ± SD; 67.12 ± 9.05 kg), and 247.1-413.5 g (mean ± SD; 335 ± 25.18 g, dry weight), respectively. The present results were comparable to previous reports. Tongboriboon[10] reported arsenic content in fruits and vegetables ranged between 0.23 and 2.97 μg/g. The Ministry of Public Health summarized that total arsenic concentration in foods and shallow well water collecting from Ronphibun area between 1990 and 2000 were ranges from > 0 to 15.35

μg/g and 0.02-5.5 mg/l, respectively.[11] Boriboon[7] collected the duplicate diet from 90 households in Ronphibun and found that daily arsenic concentration with ranges from > 0.24 to 7125.75 μg/d. When compared to the values from the data previously reported, the concentrations of arsenic in this study were relative low. This finding resulted from residents in this area avoiding using contaminated shallow well water for drinking or cooking in the last 10 years. Water and foods are major potential sources of arsenic exposure in the arsenic-affected area but it is difficult to identify the concentrations of arsenic in individual types of food in this study because the present study used replicate diet sampling for the purpose of actual intake. Further studies are needed to better understand the levels of arsenic in different types of food in the Ronphibun district.

The Student's *t*-test showed that arsenic concentrations (μg/g) were statistically different between genders (*p*-value < 0.05). Arsenic content in duplicate diets from males were higher than females, similarity to report in West Bengal, India, Bangladesh, and USA.[12-14] Daily arsenic intakes (μg/d) from male samples were statistically higher than female (*p*-value < 0.05). However, arsenic intake based on μg/kg body weight/d was not statistically different (Table 1). Estimation average dietary intakes of arsenic in this study were 297.89 μg/d for male and 252.06 μg/d for female. The average daily intake by adults in our study was higher than intake estimated previously in other countries: 47 μg in USA[14]; 59.20 μg in Canada[15]; 65 μg in UK[16]; and 62.1 μg in French.[17] The present results were quite comparable in Taiwan, Spain, Japan, and Bangladesh (Table 2).[18-22] The difference of arsenic intake was resulted from the eating habits including cooking methods, as well as arsenic concentration presented in foods from distinct geographical areas. Cooking methods vary in different countries for example, people cook rice with very little water in Japan whereas in Bangladesh, rice is cooked with excess water and water that is not absorbed during cooking is discarded. Thus, the arsenic concentration may differ from the method of cooking.[23, 24] US EPA estimates that preparing foods with arsenic containing water may increase arsenic content by as much as 10 to 30% for most foods, beans and grains that absorb water when cooked may absorb up to 200%.[25] After cooking, most of water is evaporated but arsenic contained in the initial water stays with the food and is concentrated. Data from the literature on the dietary metal intake in various countries show great variation. It is obvious that food choice is influenced by many interrelated factors, including various physiological, social, and cultural factors.

Table 1 Arsenic intake via duplicate diet of Ronphibun villagers

Parameter	Range	Mean ± SD
Arsenic concentration (μg/g)		
Male	0.51 - 1.23	0.83 ± 0.15*
Female	0.49 - 1.33	0.71 ± 0.18*
Total	0.49 - 1.33	0.78 ± 0.16
Daily Arsenic intake (μg/d)		
Male	153.36 - 550.26	297.89 ± 87.95*
Female	130.72 - 435.38	252.06 ± 51.75*
Total	130.72 - 550.26	274.97 ± 74.78
Arsenic intake (μg/kg body weight/d)		
Male	2.64 - 9.31	4.68 ± 1.56
Female	2.56 - 9.07	4.75 ± 1.20
Total	2.56 - 9.31	4.71 + 1.42

*p - value < 0.05

Table 2 Daily dietary intake of total arsenic estimated for population from different countries.

Country	Arsenic from diet (μg/d)	Reference
Bangladesh	515	13
Bangladesh	174	22
Taiwan	15-211	18
Japan	182	21
Japan	280	20
USA	47	14
Canada	59.20	15
Spain	245	19
UK	65	16
French	62.1	17
Thailand	> 0.24-7125.75	7
Thailand	274.97	Present study

The estimated daily intake of total arsenic, both male and female in the present study, on the average consumption of 335 g for individual weighing approximately 68 kg and arsenic concentration of 0.49 - 1.33 μg/g was found the daily intake in the ranges of 2.74 - 7.43 μg/kg body weight/d. The Food and Agriculture Organization/World Health Organization (FAO/WHO) established a reference value for inorganic arsenic, the provisional tolerable weekly intake (PTWI) of 142.8 μg/d for an individual weighing 68 kg (15 μg/kg body weight/week). To have a reference value on a daily basis giving the provisional tolerable daily intake (PTDI) of 2.1 μg/kg body weight/d. Based on the previous reports on inorganic arsenic in food composites[5], we assumed that about 30% of the total arsenic in this present study from duplicate diet sample is inorganic. Then, using the average food consumption both male and female in the present study of 335 g and the highest of arsenic concentration of 1.33 μg/g, we estimated the exposure of the Ronphibun residents to inorganic arsenic to be 1.97 μg/kg body weight/d, slightly lower the PTDI value. However, it is known that the arsenic and species bioavailability in food is variable. It is necessary to continue this study, in order to observe aspects related to arsenic speciation, obtain information about cooking processes that can influence the arsenic bioavailability as well as that of its species, to determine the intake accurately.

Conclusion

The estimate of the Ronphibun exposure to total arsenic via duplicate diet method showed that the concentration of arsenic was below the maximum permissible concentration limit set by Ministry of Public Health, Thailand (2 μg/g). It may be concluded that concentration of arsenic was still within acceptable level of human consumption. Comparison with earlier studies revealed that the concentration of arsenic is decreasing.

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