ASSESSMENT OF IODINE NUTRITION STATUS AMONG SCHOOL AGE CHILDREN OF NEPAL BY URINARY IODINE ASSAY

B Gelal[1], M Aryal[1], BK Lal Das[1], B Bhatta[2], M Lamsal[1] and N Baral[1]

[1]Department of Biochemistry, B.P. Koirala Institute of Health Sciences, Dharan; [2]Alliance Nepal, Lalitpur, Nepal

Abstract. The present study was undertaken to evaluate the iodine status of Nepalese school age children by measuring urinary iodine excretion (UIE). A population based cross-sectional study was conducted during November-December 2006 among 1,094 school age children. Spot urine samples were collected from all children and UIE was measured during February to March 2007 by an ammonium persulfate digestion microplate (APDM) method. The median UIE at the national level was 193.10 μg/l, indicating adequate iodine intake in Nepalese schoolchildren. The proportion of the population having UIE below 50 μg/l and below 100 μg/l were 4.5% and 22.0%, respectively. Determination of precision of the method was done following calculation of the inter- and intra-assay coefficient of variation (CV). At low, medium and high concentrations of urinary iodine the intra-assay CVs were 6.3, 1.8 and 1.9%, respectively. The inter-assay CVs for low, medium and high concentrations of urinary iodine were 11.9, 4.9 and 6.2%, respectively. Therefore, current iodine nutrition status is at satisfactory levels in Nepal. An effective monitoring program must be continued to ensure optimal iodine status and prevent the population from developing iodine deficiency disorder (IDD).

INTRODUCTION

Iodine deficiency disorder (IDD) is a global public health problem with clusters around the mountainous regions of Europe, the central Mediterranean, South and Central America, Africa and Asia (Sarkar *et al*, 2007). It is one of the oldest and most insidious of human health problems. Iodine deficiency not only causes goiters, but may also result in irreversible brain damage in the fetus and infant and retard psychomotor development in the child. Iodine deficiency is the most common cause of preventable mental retardation; it also affects a child's learning ability. Though the prevalence varies, the problem of IDD is confined to developing countries. About 1,000 million people are at risk for IDD, of which 200 million suffer from goiter, 5 million have gross cretinism with mental retardation and 15 million suffer from lesser mental defects (Sarkar *et al*, 2007). Nepal is considered an endemic area for iodine deficiency by the WHO, UNICEF and ICCIDD (2001).

The daily intake of iodine sufficient to prevent iodine deficient goiter in adults is 150 μg per day. Additional iodine is required during pregnancy and lactation (WHO, UNICEF and ICCIDD, 2001). Iodine is rapidly absorbed in the circulation in the form

Correspondence: Dr Nirmal Baral, Department of Biochemistry, BP Koirala Institute of Health Sciences, Dharan, Nepal.
Tel: 977 25 525555 ext 2463/2461/3255 (R)
E-mail: nirmalbaral@yahoo.com

of inorganic iodine, which is rapidly cleared by the kidneys. In humans, greater than 90% of iodine intake is excreted in the urine providing an estimate of current iodine intake rather than past iodine intake (Ministry of Health, 1998; Guttikoda *et al*, 2003). Urinary iodine excretion (UEI) is therefore considered a good biochemical marker of recent dietary intake of iodine and is the test of choice for evaluating the degree of iodine deficiency and its correction (WHO, UNICEF and ICCIDD, 2001).

School age children, 6-11 years old, form a useful study group for assessing iodine deficiency because of their physiological vulnerability to disease, their accessibility through school (Joshi *et al*, 2006), and as a representation of iodine deficiency in the community (International Council of Iodine Deficiency Disorders, 1999; Biswas *et al*, 2006).

Even though increased median urinary iodine excretion and decreased prevalence of IDD has been achieved by salt iodization and various public awareness programs, it has not been able to progress towards sustainable elimination of IDD as a public health problem (20% of the population having a UIE below 50 μg/l and 50% below 100 μg/l). Thus, this study aimed to determine the current urinary iodine status in Nepalese schoolchildren by measuring UIE.

MATERIALS AND METHODS

We carried out a cross-sectional population based study in which 1,094 urine samples were collected from 6-11 year school age children chosen using multi-stage cluster sampling in November and December, 2006 by a trained enumerators and analyzed in February and March, 2007 at the Department of Biochemistry, B.P. Koirala Institute of Health Sciences, Nepal. The clusters were chosen taking into consideration the three geographical regions of Nepal: mountainous, hilly and terai. From the mountainous, hilly and terai regions, 203, 433, and 458 urine samples were collected, respectively to represent the five regions of Nepal (Eastern, Central, Western, Mid-western and Farwestern).

The urine samples were collected in 10 ml plastic containers which they were half filled. The containers had screw caps to prevent leakage and evaporation and were labeled with stickers. The samples were transported to the laboratory maintaining a cold chain. The urine samples were analyzed for iodine concentration using ammonium persulfate to digest the samples (Ohashi *et al*, 2000), followed by the Sandell-Kolthoff reaction for assessing the severity of IDD, which were based on a median urinary iodine concentration below 20 μg/l indicating severe deficiency, 20-49 μg/l indicating moderate deficiency, 50-99 μg/l indicating mild deficiency, 100-199 μg/l indicating adequate intake, 200-299 μg/l indicating more than adequate iodine intake and > 300 μg/l indicating excess iodine intake (WHO, UNICEF and ICCIDD, 2001).

Urine samples, calibrators and pooled urine (50 μl each) were pipetted into the different wells of the microplate followed by the addition of 100 μl of ammonium persulphate (1.31 mol/l) solution. The microplate (96-well Microplate, Tarson, India) was set in a sealing cassette (Hitachi Chemical Techno-Plant, Japan), closed tightly and kept for 60 minutes in an oven at 110ºC. After digestion, 50 μl of digested aliquot was transferred to the corresponding wells of another 96-well microplate. One hundred μl of arsenious acid solution (0.05 mol/l) was added followed by 50 μl of ferric ammonium persulphate (0.019 mol/l) within one minute by using a multi-channel pipette (Microlit, MC-8/300) as described by Ohashi *et al* (2000). A 96-well microplate was al-

Table 1
Median urinary iodine excretion in geographical and development regions of Nepal.

Regions	Location	Median UIE (μg/l)
Geographic	Terai (*n* = 458)	214.4
	Hilly (*n* = 433)	208.9
	Mountains (*n*= 203)	126.5
Developmental	Eastern (*n* = 282)	216.6
	Central (*n* = 291)	210.7
	Western (*n* = 174)	210.4
	Midwestern (*n* = 169)	178.6
	Farwestern (*n* = 178)	132.6

Table 2
Iodine nutrition status in three geographical regions of Nepal.

Iodine status	Terai (*n* = 458)	Hilly (*n* = 433)	Mountain (*n* = 203)	Total (*n* = 1,094)
Severe deficiency (< 20 μg/l)	2 (0.2%)	6 (0.5%)	12 (1.1%)	20 (1.8%)
Moderate deficiency (20-49 μg/l)	10 (0.9%)	11 (1.0%)	9 (0.8%)	30 (2.7%)
Mild deficiency (50-99 μg/l)	71 (6.5%)	63 (5.8%)	57 (5.2%)	191 (17.5%)
Optimal (100-199 μg/l)	136 (12.4%)	133 (12.2%)	76 (6.9%)	345 (31.5%)
More than adequate (200-299 μg/l)	123 (11.2%)	115 (10.5%)	33 (3.0%)	271 (24.8%)
Excessive iodine intake (> 300 μg/l)	116 (10.6%)	105 (9.6%)	16 (1.5%)	237 (21.7%)

lowed to stand for 30 minutes at 25ºC and absorbance was measured at 405 nm with an ELISA reader (Merck Mios Junior, Germany).

The urinary iodine level was not normally distributed thus the results were expressed as a median. The prevalence was expressed as a percentage. The chi-square test was also applied. Data were analyzed using SPSS version 11.5.

RESULTS

The median urinary excretion from all the regions combined was 193.1 μg/l indicating adequate iodine intake. Median urinary iodine concentration in the terai and hilly regions were 214.4 μg/l and 208.9 μg/l, respectively, indicating more than adequate iodine intake. However, the median urinary iodine concentration in the mountainous region was lower than optimal at 126.5 μg/l (Table 1).

In the different regions the levels were: Eastern (216.6 μg/l), Central (210.7 μg/l) and Western (210.4 μg/l) but lower levels were found in the Midwestern (178.6 μg/l) and Farwestern (132.6 μg/l) regions (Table 1).

The iodine nutrition results from the three geographic regions of Nepal are given in Table 2 and the iodine status in development regions are shown in Table 3. Twenty-two percent of the population had a median

Table3
Iodine status in five developmental regions of Nepal.

Iodine status	Eastern (*n* = 282)	Central (*n* = 291)	Western (*n* = 174)	Mid western (*n* = 169)	Far western (*n* = 178)	Total (*N* = 1,094)
Severe deficiency(< 20 µg/l)	1 (0.1%)	4 (0.4%)	3 (0.3%)	3 (0.3%)	9 (0.8%)	20 (1.8%)
Moderate deficiency (20-49 µg/l)	2 (0.2%)	6 (0.5%)	2 (0.2%)	8 (0.7%)	12 (1.1%)	30 (2.7%)
Mild deficiency (50-99 µg/l)	47 (4.3%)	60(5.5%)	16 (1.5%)	26 (2.4%)	42 (3.8%)	191 (17.5%)
Optimal (100-199 µg/l)	89(8.1%)	63 (5.8%)	76 (6.9%)	53 (4.8%)	64 (45.9%)	345 (31.5%)
More than adequate (200-299 µg/l)	72 (6.6%)	76 (6.9%)	44 (4.0%)	47 (4.3%)	32 (2.9%)	271 (24.8%)
Excessive iodine intake (> 300 µg/l)	71 (6.5%)	82 (7.5%)	33 (3.0%)	32 (2.9%)	19 (1.7%)	237 (21.7%)

Table 4
Intra- and inter-assay CV among three urine samples.

Urine sample	*N*	Intra-assay		Inter-assay	
		Urinary iodine (µg/l) (Mean ± SD)	CV (%)	Urinary iodine (µg/l) (Mean ± SD)	CV (%)
Low	10	23.8 ± 1.5	6.3	30.1 ± 3.6	11.9
Medium	10	97.6 ± 1.8	1.8	95.3 ± 3.9	4.1
High	10	370 ± 7.1	1.9	402 ± 25.12	6.2

UIE below 100 µg/l which shows some degree of iodine deficiency. Of these 1.8% (*n* = 20) were severely deficient, 2.7% (*n* = 30) were moderately deficient and 17.5% (*n* = 191) were mildly iodine deficienct.

The intra- and inter-assay coefficient of variation (CV) of the medium urinary iodine was less than the low and high urinary iodine values. The intra-assay CVs for the low, medium and high urinary iodine levels were 6.3, 1.8 and 1.9%, respectively. The inter-assay CV for the low, medium and high urinary iodine values were 11.9, 4.1 and 6.2%, respectively (Table 4).

DISCUSSION

The average total national median urinary iodine excretion level was 193.1 µg/l indicating adequate iodine intake according to WHO, UNICEF and ICCIDD (2001). In Nepal, goiter prevalence survey in 1966 indicated 55% of the population had goiter. A 1969 survey in Trisuli and Jumla indicated 74-100% of schoolchildren were goiterous with cretinism and had deaf mutism (Krishan *et al*, 2000). In 1973 a salt iodization program was initiated (Schulze *et al*, 2003). The IDD surveys in 1986 and 1992 showed a greater than 10% prevalence of IDD in endemic areas of Nepal. The median urinary iodine concentrations in Nepal at the national level iodine status survey was 70 µg/l in 1992 and 143.8 µg/l in 1998 according to the Nepal Micronutrient Status Survey (Ministry of Health, 1998), whereas the value was 188 µg/l according to the Nepal Iodine Deficiency Disorders Status Survey in 2005. The prevalence decreased from 35.0% in 1998 to 27.4% in 2005. This result showed an increasing median urinary iodine compared to a previous Nepal Micronutrient Status Survey

(NMSS) in 1998 (143.8 μg/l) and a Nepal Iodine Deficiency Disorder Status Survey in 2005 (188.0 μg/l). In this study, the proportion of the population with low median UIE values below the WHO cut-off value of 100 μg/l was 22.0%, which is significantly lower than the Nepal Micronutrient Status Survey in 1998 (35.1%) and the Nepal Iodine Deficiency Disorders Status Survey in 2005 (29.5%).

The WHO, UNICEF and ICCIDD joint criteria state iodine deficiency is not a major public health problem in a population when the median UIE is 100-199 μg/l, when not more than 20% of urine samples have a UIE level less than 50 μg/l and not more than 50% of urine samples have UIE level less than 100 μg/l. This was met in all three surveys and decreased significantly from 13.6% (Nepal Micronutrient Status Survey, 1998) and 9.5% (Nepal Iodine Deficiency Disorders Status Survey 2005) to 4.5% in our present study.

Over all, the geographical regions the median urinary iodine was found to be in normal, was elevated in Terai (182.7 to 214.4 μg/l) and in the hilly region (204.0 and 208.9 μg/l) but decreased in the mountainous region (164.6 to 126.5 μg/l) as compared to the Nepal Iodine Deficiency Disorders Status Survey of 2005. The low level of median UIE in the mountainous region may be because of less accessibility of packaged iodized salt, leaching of iodine from the soil by snow and rainfall and a lack of knowledge regarding the use of iodized salt. The successful intervention program has not been influenced by seasonal variation. According to Schulze *et al* (2003) the maximum seasonal excretion urinary iodine excretion occurred in April to June and was fairly constant during the other months.

The most common presentations of IDD in this population are hypothyroid or euthyroid goiter. Hypothyroidism is one of the consequences of iodine deficiency. This indicates the persistence of iodine deficiency in the population (Baral *et al*, 2002). Thyroid hormone levels which can diagnose iodine deficiency were not included in the present study due to cost.

Nepal is progressing towards sustainable elimination of IDD. Severe cases of iodine deficiency are still present in the population. Use of iodized salt and its accessibility in remote areas has improved the iodine status in the population. An effective monitoring programs along with health awareness programs must be continued to ensure the population has optimal iodine status and to prevent them from developing iodine induced hyperthyroidism and autoimmune thyroid disease.

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