ASSAY OF IODINE DEFICIENCY STATUS IN THREE ECOLOGICAL REGIONS OF NEPAL BY A MICRODIGESTION METHOD

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Abstract. Iodine deficiency disorders (IDD) are a global public health problem. In continuation of the efforts to eliminate the iodine deficiency in different parts of the world, IDD surveys are being conducted to assess the status of iodine nutriture. A survey was conducted in Nepal in 1998 with assistance from UNICEF. We present the status of the iodine nutriture, as assessed from urinary iodine levels of casual samples by a micro-digestion method, in the three ecological regions: Terai (flat region), Hilly region (300-3,000 m altitude) and mountainous regions (>3,000 m altitude) of Nepal. Terai region is more affected, having iodine deficiency in 18.6% of the population. The hilly and mountainous regions were found to have 11.2% and 9% iodine deficient populations respectively. The study shows improvement in iodine deficiency status with respect to previous surveys yet it continued to be prevalent in the country as a major public health problem which requires strengthening of preventive measures.

INTRODUCTION

Iodine deficiency disorders (IDD) are most common preventable cause of mental handicap in the world. Iodine deficiency causes adverse affects on all stages of human growth and development including fetus, neonates, children, adolescents and the adult population. The spectrum of IDD has been well reviewed by Hetzel *et al* (1987).

Disorders arising from severe and chronic dietary iodine deficiency are recognized as a global public health problem today. The global elimination of IDD by iodine supplementation is an attainable goal. WHO assembly, 1990, the World Summit for Children 1990, and UNICEF, passed a resolution accepting the goal of elimination of IDD by the year 2000 (Hetzel, 1994). Worldwide 1.56 billion people are at risk of IDD, 834 million are goitrus and 20 million people are suffering from varying degree of mental retardation caused by iodine deficiency (WHO, 1998). Southeast Asia (including Nepal, Bhutan, India, Indonesia, Myanmar, Thailand) is estimated to have the largest share of IDD (486 million at risk and 176 million goitrus) (WHO, 1992; WHO/UNICEF/ICCIDD,1993).

In Nepal, the country wide goiter prevalence survey in 1965-6 indicated that 55% of the population had goiter representing all the three geographical regions of country: Mountains (>3,000 m), Hilly (300 to 3,000 m) and the low flat Terai region (Hetzel, 1994). In 1969 a survey in Trishuli (550 m) and Jumla (2,250 m), indicated 74-100% school children were goitrous associated with cretinism and deaf mutism (Stanbury and Pinchera, 1994; Valix, 1976). The IDD surveys in 1986 and 1992 showed greater than 10% of IDD prevalence in endemic areas of Nepal (Achary, 1991; Pandav, 1994).

There are various markers to assess the level of adequate iodine nutriture. Estimation of iodine content in urine is a widely used laboratory marker to asses the iodine nutriture. In the human, greater than 90% of the iodine usually is excreted in the urine. A 24 hour collection of urine sample is a most reliable marker. But in view of the practical problems, the measurement of iodine concentration in a casual sample is considered as an excellent surrogate (Dunn *et al*, 1993) . For mass screening, urinary iodine excretion (UIE) of more than 100 µg/l reflects an adequate iodine nutriture in a defined population. UIE below this level indicates biochemical iodine deficiency.

In view of the above, a comprehensive survey of urinary iodine status in Nepal was conducted in 1998 with assistance from UNICEF and New Era, Kathmandu, to assess the current iodine nutriture status. Here, we present the distribution analysis of the urinary iodine levels in the three ecological zones of the country.

MATERIALS AND METHODS

Sampling

Urine samples were collected according to the

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Table 1
Urinary iodine concentrations (μg/l) measured in casual samples of people in different ecological regions of Nepal.

Ecological region	Mean iodine concentration ± SD (Median)
Terai (Plain area)	230.5±149(218.4)
Hills	281.0±137(291)
Mountains	289.1±180(304.4)
Nepal (over all)	263.5±148(262.9)

methodology recommended by Joint WHO/ UNICEF/ ICCIDD consultation on Iodine deficiency disorder indicators (WHO/UNICEF/ICCIDD, 1993). The study population included throughout Nepal. All 20 districts of Terai region were grouped from east to west into five strata. Each stratum had nearly 600 samples of urine collected randomly, giving a total of 2,999. The hilly region (39 districts) were similarly divided into five strata. A total of 2,999 samples were collected in a similar manner as above. The mountainous region (16 districts) which has less population density were grouped into three strata from east to west. A total of 1,799 samples (approximately 600 from each) were collected. Adding all the three ecological regions a total came to 7,797 samples covering all 75 districts of Nepal.

Urinary iodine analysis

The iodine content of urine samples was estimated according to the procedure recommended by UNICEF (Dunn *et al*, 1993) after minor modification of digestion process. Instead of using perchloric acid, the urine sample was digested with 30% ammonium persulfate (APS) in a specially designed digestion apparatus (Hitachi, Japan). Fifty μl of urine sample was taken in microtiter plate with 100 μl of APS and digestion was carried out at 110°C for 75 minutes. Fifty μl of each digested sample was transferred to a new microtiter plate and to it 100 μl of arsenious acid solution (prepared by dissolving 10 g arsenious trioxide and 25 g NaCl/l in lN H_2SO_4) was added and left for 15 minutes. To the above 50 μl of cerric ammonium sulfate solution (48 g/l in 3.5% H_2SO_4) was added. The iodine content was quantitatively estimated spectrophotometrically at 408 nm using ELISA reader (Stat fax 2000, USA) by its property of reducing the yellow colored Ce^{4+} ions to colorless Ce^{3+} ions. The amount of iodine content was expressed in μg/l. The validation of urinary iodine estimation was checked internally by using internal quality control and externally by external quality control at the ICCIDD, New Delhi.

Statistical analysis

Chi-square test was applied to asses the difference in prevalence of iodine deficiency in different ecological regions. A p-value of < 0.05 was considered as the level of significance.

RESULTS

The urinary iodine concentrations in casual samples of people living in different ecological regions are given in Table 1. In all the three ecological regions the mean urinary iodine concentrations were found to be within normal levels. But the prevalence of iodine deficiency in over all Nepal in the present survey was found to be 13.52%. The prevalence of iodine deficiency in population of different ecological regions of Nepal is shown in Table 2. In Terai region districts, 18.9% people were found to have urinary iodine level less than 100 μg/l. In hilly region districts 11% of sample population were found to be deficient in iodine nutriture, whereas in the mountainous region (>3,000 m) districts, 8.8% samples had iodine deficiency.

Based on the levels of urinary iodine, the iodine deficiency had been classified into mild (urinary iodine 51-100 μg/l), moderate (21-50 μg/l) and severe (0-20 μg/l). Table 3 describes the distribution of each class in different areas. Terai region had the highest number of severely iodine deficient people (7.8%) in comparison to hills (4.27%) and mountain regions (3.06%). Distribution of moderately iodine deficient people are found nearly similar in the three regions - Terai (3%), hills (2.4%) and mountain (2.6%). Proportion of mildly deficient people were also highest in Terai (8.07%), in comparison to hills (4.3%) and mountain (3.2%). The prevalence in three ecological regions were found to be significantly different ($p<0.002$) from each other.

DISCUSSION

The modified method of urinary iodine assay used in the present study was found to be fairly reproducible and accurate. This method required a very small quantity of samples and reagents. There is no need of fuming cupboard and improved ex-

Table 2
Prevalence of iodine deficiency in different ecological regions of Nepal.

Ecological region	Prevalence of iodine deficiency		
	IDD (Urinary iodine ≤ 100 μg/l)	Normal (Urinary iodine > 100 μg/l)	Total
Terai (plain area)	18.9% (566)	81.1% (2,433)	100% (2,999)
Hills	11% (329)	88% (2,670)	100% (2,999)
Mountains	8.8% (159)	91.2% (1,640)	100% (1,799)
Nepal (over all)	13.52% (1,054)	86.48% (6,743)	100% (7,797)

Figures in parenthesis indicate actual number of subjects.

Table 3
Grades of severity of Iodine deficiency in different ecological regions of Nepal.

Ecological region	Urinary iodine (μg/l)			
	Severe 0-20	Moderate 21-50	Mild 51-100	Total
Terai (plain area)	7.8% (234)	3% (90)	8.07% (242)	18.9% (566)
Hills	4.27% (128)	2.4% (72)	4.3% (129)	11% (329)
Mountains	3.06% (55)	2.6% (46)	3.2% (58)	8.8% (159)
Nepal (Over all)	5.35% (417)	2.7% (208)	5.5% (429)	13.52% (1,054)

Figure in parenthesis indicate actual number of subjects.

haust system in the laboratory. The use of an ELISA reader for taking the absorbance and provision of processing nearly hundred samples at a time has made it rapid. The validity of this microdigestion method was checked (data not shown) and found to be very suitable for urinary iodine assay in large scale.

The present survey of iodine excretion in population of Nepal was carried out from casual samples for its practicability. The samples were collected from all the districts of the country. As evident from the results, the mean concentrations of urinary iodine over all Nepali population was found to be 263.5 ±148/1 and similar levels in all the three ecological regions viz Terai (230.5 ±149), hills (281±137) and mountains (289.1±180), which falls within the normal levels. Such wide survey of urinary iodine levels covering all parts of the country is the first time in Nepal. This solved the problem of getting a nationwide base line data in this regard. The previous nation wide survey conducted to assess the IDD problem in Nepal in 1965-6 was based on goiter survey. The urinary iodine assay being more sensitive method than goiter survey (Pardede *et al*, 1998) will be indicative of the extent and gravity of the problem.

There are many reports available which shows that Nepal is a country having IDD in the endemic form and it is distributed in all ecological areas of the country (Pandav, 1994b). Because of variation in study group selection, sample size, geographical areas included, the method and criteria used, year and other reasons, the prevalence of IDD was found to vary from study to study (Pandav, 1994a; Ratcleiffe *et al*, 1991; Morata *et al*, 1976; Ibberston, 1974; McKinnon, 1968). The gravity of the problem being most serious in Jumla and Trisuli has drawn attention of the many investigators (Pandav, 1994a, b; Stanbury and Pinchera, 1994). However, in all the reports, the prevalence of IDD was found to be very high, and was indicated as a great public health problem in Nepal which needed immediate attention. Various measures like supply of iodized salt throughout the country and injection of iodized oil in mountain and hilly regions (Pandav, 1994a, b); Subramanian, 1990) has been employed during last

15 years to address the problem.

Our study shows the overall prevalence of iodine deficiency in Nepal to be 13.52% with 5.35% of the population affected with severe iodine deficiency (urinary iodine below 20 μg/l) demanding immediate attention. The Terai having the highest population density and 7.8% of them being sufferers of severe deficiency indicate the magnitude of the problem. Mountainous regions are more prone to iodine deficiency because of leaching out of the iodine from the soil compared to hills and Terai. But the present study revealed that the magnitude of iodine deficiency is more severe in Terai than in hills and mountains. This is probably because of implementation of injection of iodized oil in hills and mountain regions, and the only preventive measure in Terai region is supply of iodized salt which has its inherent demerits (Hetzei and Clugston, 1994). Other reasons might be the stringency in supply of iodized salt in hills and mountains but not in the Terai, and the easy availability of non-iodized salt in the Terai region through open border with India.

The present study also shows that there is a significant improvement in the iodine deficiency status in Nepal in comparison to 1965-6 survey data. As per prevalence wise classification (Hetzel and Clugston, 1994), Nepal has entered into mild IDD grade (prevalence 5-20%) from severe IDD prevalence (30% or more). Even the prevalence of iodine deficiency in Jumla and Trisuli has reduced to 7.5% (mild 3.75%, moderate 1.25% and severe 2.5%) and 11% (mild 4%, moderate 3% and severe 4%) respectively. But IDD is continued to be prevalent as a public health problem in Nepal till date. Problem being of great magnitude, with a large proportion of the population belonging to severely deficient group, it calls for strengthening of existing preventive measures, monitoring iodine content of salt supplied to the population, repeated surveys and warrants a strict implementation of ameliorative measures. Other measures like implementation of iodized oil injection in Terai region, fortification of bread and/or wheat flour with iodine, ban on sale and unauthorized import of non-iodized salt and supplementation of iodine through water irrigation system may be considered based on practicability.

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