

Household Salt Iodine Content Estimation with the Use of Rapid Test Kits and Iodometric Titration Methods

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ABSTRACT

Background: Universal salt iodization remains the best strategy for controlling iodine deficiency disorders in Nepal.

Aims: This study was designed to study the salt types and the household salt iodine content of school aged children in the hilly and the plain districts of eastern Nepal.

Material and Methods: This cross-sectional study was carried out on school children of seven randomly chosen schools from four districts, namely, Sunsari, Dhankuta, Sankhuwasabha and Tehrathum of eastern Nepal. The school children were requested to bring two teaspoonfuls (approx. 12-15 g) of the salt which was consumed in their households, in a tightly sealed plastic pouch. The salt types were categorized, and the salt iodine content was estimated by using rapid test kits and iodometric titrations. The association of the salt iodine content

of the different districts were tested by using the Chi-square test. The sensitivity, specificity, positive predictive values, and negative predictive values of the rapid test kits were compared with the iodometric titrations.

Results: Our study showed that mean \pm SD values of the salt iodine content in the four districts, namely, Sunsari, Dhankuta, Sankhuwasabha and Tehrathum were 34.2 \pm 17.9, 33.2 \pm 14.5, 27.4 \pm 15.1 and 48.4 \pm 15.6 parts per million (ppm). There were 270 (38.2%) households which consumed crystal salt and 437(61.8%) of the households consumed packet salts.

Conclusions: Our study recommends a regular monitoring of the salt iodization programs in these regions. More families should be made aware of the need to ensure that each individual consumes iodized salt.

Key Words: Iodine deficiency, Salt iodine content, Nepal

INTRODUCTION

Iodine deficiency disorders (IDD) have multiple adverse effects on the growth and development in animals and humans and they are a most preventable cause of brain damage [1-3]. The iodization of salt, a common food which is used by a majority of the population, is a proven intervention for the prevention of IDD [4,5]. The sustainable elimination of IDD is a near approaching goal in many countries, with the access of the iodized salt to be 71%, up from 20% in 1990. Since 1993, the World Health Organization (WHO) has initiated the global primary intervention strategy for Universal Salt Iodization (USI), to control iodine deficiency disorders in the developing countries, which also includes Nepal. National surveys which were carried out in 1998, 2005 and 2007 have shown a sustainable improvement towards the iodine nutrition in Nepal, with urinary iodine levels of 143.8 μ g/L, 188 μ g/L and 202.8 μ g/L and adequate salt iodine contents (>15 ppm), of 55.2%, 57.7% and 77% in the three consecutive years respectively [6-8]. The WHO/UNICEF/ICCIDD recommends that the salt iodine content at the packaging level must be 50-60 ppm and 20-30 ppm at the retail shops, to achieve at least 15 ppm in the household dietary consumption [9]. Salt iodine content is one of the indicators which is used for a sustainable elimination of iodine deficiency as a public health problem, followed by a median urinary iodine concentration (UIC) of the population and programmatic indica-

tors at the national and regional levels [4,9]. The availability and the usage of adequately iodized salt (>15 ppm and <40 ppm at household level) must be ensured. This is demonstrated by its consumption by more than 90% of households in a given population. The conditions which demonstrate a successful usage of iodized salt in households for the elimination of an iodine deficiency are: i) 95% of salt at the households must be iodized (>15 ppm and <40 ppm) when they are estimated with the salt iodine titration and they should be greater than or equal to 90% when they are estimated by the rapid test kits (RTK). The median UIC should be in the range of 100-199 μ g/l, with pregnant women having 150-249 μ g/l and the recent monitoring data should be within the last five years. The WHO/UNICEF/ICCIDD recommends that iodine must be added at a concentration of 20-40 mg iodine per kg salt, which is dependent on the local salt intake. Potassium iodide or potassium iodates are the two forms, in which iodine can be added to the household salt [3,9].

The salt iodine content is estimated by iodometric titrations and rapid test kit methods. Iodometric titration gives the exact value of iodine in the salt in ppm, whereas the rapid test kits give a semi-quantitative measure of the iodine content [4,9]. This study was designed to assess the salt types and the household salt iodine content of school aged children of the hilly and plain districts of the eastern region of Nepal.

MATERIALS AND METHODS

Study Design and the Subjects

This cross-sectional study was carried out in the Department of Biochemistry, B. P. Koirala Institute of Health Sciences (BPKIHS), Dharan, Nepal, from August 2009 to July 2010. A convenient sampling was performed in seven randomly chosen schools from four districts, namely, Sunsari, Dhankuta, Sankhuwasabha and Tehrathum of the eastern region of Nepal. Sunsari lies in the plains, whereas Dhankuta, Sankhuwasabha and Tehrathum lie in the hilly regions of eastern Nepal. A total of 707 school aged children who were 6-12 years of age were enrolled for the study, after getting written consents from their guardians and school principals. This study was approved by the Institutional Ethical Review Board (IERB), BPKIHS.

Data Collection

The school children were requested to bring two teaspoonfuls (approx. 12-15 g) of the salt which was used in their households, in a tightly sealed plastic pouch. The salt types were categorized and the salt iodine content was estimated by using rapid test kits (MBI Kits, India) and iodometric titration methods [4,9]. The accuracy and the precision of the methods were calculated prior to the assay. A known standard of salt iodine which contained 50 ppm iodine was periodically measured throughout the analysis, by both the methods. The intra-assay and the inter-assay coefficients of variation (CV) for the iodometric titration were 2.8% and 6.9% respectively.

Inclusion Criteria: School children who were six to twelve years of age and whose guardians were willing to give their consents, were enrolled for the study.

Exclusion Criteria: School children who were aged less than six years and more than twelve years and whose guardians were not able to provide written consents were excluded from the study.

The Salt Iodine Content Estimation

The Salt Iodine Content as Measured by the Rapid Test Kits

The semi-quantitative estimation of the salt iodine by using a rapid test kit (RTK) is based on the reaction between starch and iodine to form starch-iodine complex. This test solution contains an acidic buffer and a reducing agent, which convert potassium iodate (KIO_3) to elemental iodine (I_2). This elemental iodine reacts with the iodide ion (I^-) to form a tri-iodide anion (I_3^-) and this further reacts with it to give a penta-iodide anion (I_5^-). This penta-iodide anion (I_5^-) forms a visible blue-black complex with the amylose of the starch. The colour of the test sample is compared with the standard colour

chart (<15 ppm or >15 ppm) for calculating the salt iodine content [5,9].

The Salt Iodine Content which was Measured by the Iodometric Titration

The salt iodine content is estimated in ppm by the iodometric titration method. First, the liberation of free iodine from the salt is carried out by adding concentrated sulfuric acid (H_2SO_4) which liberates the free iodine from the iodate in the salt sample. Excess potassium iodide (KI) is added to solubilize the free iodine, which is quite insoluble in pure water under normal conditions. Free iodine is consumed by sodium thiosulfate in the titration step. The amount of thiosulfate which is used is proportional to the amount of free iodine which is liberated from the salt. Starch is added as an external (indirect) indicator of this reaction, and it reacts with the free iodine to produce a blue colour. When it is added towards the end of the titration (that is, when only a trace amount of free iodine is left) the loss of the blue colour, or the endpoint, which occurs with a further filtration, indicates that all the remaining free iodine has been consumed by the thiosulfate [4,5].

Calculation: Iodine mg/kg (ppm) = titration volume in ml x (21.15) x normality of sodium thiosulfate x 1000/ salt sample weight in gram.

STATISTICAL ANALYSIS

The data were analyzed by using Excel 2003 and the R 2.8.0 Statistical Package for the Social Sciences (SPSS) for Windows, version 16.0 (SPSS Inc; Chicago, IL, USA). The association of the salt iodine content of the different districts by RTK method and iodometric titration method were tested by using the Chi-square test and student 't' test respectively. The sensitivity, specificity, positive predictive values, and the negative predictive values of the rapid test kits were calculated by using Analyse-it for Microsoft Excel v2.20. A p-value of <0.05 (two-tailed) was used to establish the statistical significance at the 95% confidence intervals.

RESULTS

Among the 707 salt samples which were collected from the households of the school children, the mean±SD values of the salt iodine in ppm in the four districts, namely, Sunsari, Dhankuta, Sankhuwasabha and Tehrathum were found to be 34.2±17.9, 33.2±14.5, 27.4±15.1 and 48.4±15.6 ppm respectively [Table/Fig-1]. There were 270 (38.2%) households which consumed the crystal salt and 437 (61.8%) households consumed the packet salt in the four districts [Table/Fig-1]. The household salts which had <15 ppm iodine in the four districts were 123 (17.3%) and those which had >15 ppm iodine were 584 (82.6%). Between the two

Characteristics		Districts				p- value
		Sunsari	Dhankuta	Sankhuwasabha	Tehrathum	
Salt type	Open	50(26.2%)	102(52.3%)	89(65.9%)	29(15.6%)	<0.001
	Packet	141(73.8)	93(47.7%)	46(34.1%)	157(84.4%)	
SIC Rapid test kit	Zero ppm	5(2.6%)	5(2.5%)	2(1.5%)	0(0)	<0.001
	<15 ppm	34(17.8%)	12(6.2%)	87(64.4%)	28(15.1%)	
	>15ppm	152(79.6%)	178(91.3%)	46(34.1%)	158(84.9%)	
SIC titration (ppm)	Mean±SD	34.2±17.9	33.2±14.5	27.4±15.1	48.4±15.6	<0.001
	<15	46(24.1%)	35(17.9%)	32(23.7%)	10(5.4%)	<0.001
	>15	145(75.9%)	160(82.9%)	103(76.3%)	176(94.6%)	

[Table/Fig-1]: Salt types and salt iodine content (SIC) of hilly and plain districts of Eastern Nepal

Rapid Test kit	Iodometric titration			Total
	0 ppm	<15 ppm	>15 ppm	
0 ppm	0	11(1.5%)	1(0.2%)	12 (1.7%)
< 15 ppm	0	73(10.4%)	88(12.4%)	161 (22.8%)
>15 ppm	0	39(5.5%)	495(70.0%)	534(75.5%)
Total	0	123(17.4%)	584(82.6%)	707 (100%)

Table/Fig-2: Comparison of salt iodine content by iodometric titration and rapid test kit methods

districts categories, the hilly districts showed 439 (85.07%) and the plain districts showed 145 (75.9%) adequately iodized salts (>15 ppm). Our study showed a significant difference in the salt types of the four districts ($p < 0.001$). Significant differences were observed between the salt iodine contents of the four districts by the RTK ($p < 0.001$) and the salt iodine titration ($p < 0.001$) methods [Table/Fig-1]. The salt iodine content estimated by the RTK method showed 12 (1.7%) household salt samples to have no iodine at all, i.e. zero ppm, 161 (22.8%) households to have less than 15 ppm of iodine and 534 (75.5%) households to have greater than 15 ppm of iodine in the four districts. The iodometric titration showed 123 (17.4%) of the households to have less than 15 ppm of iodine and 584 (82.6%) households to have greater than 15 ppm of iodine in the four districts [Table/Fig-1]. Among the 12 samples which were shown to have zero ppm of iodine by the RTK method, 11 were shown to have < 15 ppm of iodine, whereas 1 was shown to have > 15 ppm of iodine by titration. The number of salt samples that showed inadequate iodization, i.e. < 15 ppm of iodine by the RTK method were 161, but the titration method showed that only 73 among them had < 15 ppm of iodine and that the remaining 88 salt samples had > 15 ppm of iodine. The RTK method showed that 534 salt samples had > 15 ppm of iodine, but the titration method, in contrast, showed that among these samples, only 495 had >15 ppm of iodine and that the remaining 39 had <15 ppm of iodine [Table/Fig-2]. The rapid test kits method showed a sensitivity of 84.8% (82.0 – 88.0), a specificity of 68.3% (59.0 – 77.0), a positive predictive value of 92.7% (92.0 – 94.0) and a negative predictive value of 48.6% (40.0 – 57.0) as compared to the values of the iodometric titration respectively.

DISCUSSION

Our study showed that most of the households were using packet salt, which was adequately iodized. Sunsari 141(73.8%) and Tehrathum 157(84.4%) districts showed a higher packet salt usage. Where as, Sankhuwasabha 89(65.9%) and Dhankuta 102 (52.3%) districts showed higher amounts of crystal salt distribution among the households. Packet salt is the salt which is distributed by the Salt Trading Corporation, which contains adequate amount (>30 ppm) of iodized salt which is intended to be used in households. Crystal salt is the salt which is used for livestock consumption and it does not contain adequate iodized salt. The usage of crystal salt in the Dhankuta and the Sankhuwasabha districts corresponded to the usage of salt which was intended for livestock consumption, which usually contained low contents of iodine. However, there was an increasing trend to use packet salt for household consumption, which was supported by our findings and other studies [10-12].

The salt iodine content which was estimated by the rapid test kit and the salt iodine titration of the household salts showed comparable results. The rapid test kits are used for the field-

testing purposes of the household salts when a large number of salt samples need to be estimated in populations. This is considered to be suitable for the salt iodine content estimation in community surveys [3,9]. In our study, the rapid test kits showed 84.8% sensitivity and 68.3% specificity, as compared to those of the iodometric titration. In a previous study which was conducted by Pandav et al., in India, the sensitivity of the rapid test kit was found to be 93.3% and its specificity was 90.4%. The positive predictive value was 88.2% while the negative predictive value was 94.6% [10]. Our study showed a comparable positive predictive value (92.7%), but the negative predictive value of the kits was comparably low (48%) in our study, which may have been due to the difference in the reference intervals of the RTKs which were used in our study (i.e. <15 ppm and >15 ppm). The sensitivity (84.8%) and the specificity (68.3%) were comparable; a slightly lower specificity may have been seen due to the negative predictive value and the storage of the salt samples. Joshi et al., showed that in the mid-western region of Nepal, among the 1803 salt samples which were analyzed for the iodine estimation, 289 (16.0%) were found to have less than 15 ppm of iodine and 637 (35.3%) salt samples had 15-30 ppm of iodine. Iodine levels of 30-50 ppm were found in 649 (36.0%) samples and 228 (12.6%) salt samples had iodine levels which were greater than 50ppm [13]. Ranganathan et al., reported that the iodine contents which were measured in the iodized salts by the iodometric titration showed good reproducibility, consistency and agreement between the duplicate samples [14].

The factors which influence the stability of iodine in the household salts include the duration of the storage, the size of the crystals, impurities and moisture, the ambient temperature of the storage and the humidity and the sunlight exposure [3,4,14]. In a study which was conducted by Schulze et al., in the Sarlahi District in central Nepal, they reported that among all the households which were studied, 94% had salt with some degree of iodization (7 ppm) and that 64% had 30 ppm of iodine in their salt. They also reported that adequate iodized salt was lacking in the months of August-October and that the median urinary iodine levels were low in these months. As the proportion of the households which used adequately iodized salt increased, the median urinary iodine also improved [15].

In a study which was conducted by Shakya et al., in the hilly and the plain districts of the eastern region of Nepal, the percentages of the school children who consumed adequately iodized salt were (>15 ppm) 84.6% and 98.6% respectively [16]. This finding was supported by present study, which showed that the adequately iodized salt consumption in the hilly districts was 85.1% and that in the plains, it was 75.9%. Our study included only one plain region and three hilly regions and the urinary iodine concentration, which is a population marker, was not included in this study. Further studies must be conducted at the national and the regional levels for the sustainable elimination of the iodine deficiency.

CONCLUSIONS

The urinary iodine concentration is a population measure of the iodine deficiency. Also, the salt iodine content of the household salts should be regularly monitored to know the fortification status of the iodine in the salt. As salt iodine is used as a universal vehicle for the salt iodization, it is essential to know the levels of iodine in ppm in the household salts [4]. The rapid test kits showed com-

parable results but they had variable specificities and negative predictive values. So, their primary use is in field studies, when a large number of salt samples need to be analyzed in a population. The use of the iodometric titration is recommended for the laboratory analysis of the salt iodine content. There was an increased coverage on the iodized salt in the eastern region of Nepal, as was shown by previous surveys which were done by the government and our study, which demonstrated a progress towards a sustainable elimination of the iodine deficiency. More awareness campaigns regarding the usage and the practice of iodized salts are required to be conducted by governmental agencies and family doctors, to ensure the consumption of iodized salt for the elimination of the iodine deficiency in these regions.

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