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Correlation of Iodine content of mother's milk and urine with their child's TSH level

Shruti Singh[1], Madhab Lamsal[2], Nirmal Baral[3], Nisha Keshary Bhatta[4], Dhruba K Uprety[5]

[1]Lecturer, Department of Biochemistry, B.P. Koirala Institute of Health Sciences, Dharan, Nepal, [2]Professor, Department of Biochemistry, B.P. Koirala Institute of Health Sciences, Dharan, Nepal, [3]Professor and Head, Department of Biochemistry, B.P. Koirala Institute of Health Sciences, Dharan, Nepal, [4]Professor, Department of Paediatrics and Adolescent Medicine, B.P. Koirala Institute of Health Sciences, Dharan, Nepal, [5]Professor, Department of Gynaecology & Obstetrics, B.P. Koirala Institute of Health Sciences, Dharan, Nepal

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ABSTRACT

Background: Iodine deficiency is still a significant public health problem. In the rural plains of Nepal, it remains a mild-to-moderate public health problem among pregnant and lactating women despite the availability of iodized salt. To date, only limited attention has been paid to breast-milk iodine content despite its importance in the intellectual development of infants. **Objectives:** (i) To determine iodine content in mother's urine, mother's milk and to measure their respective child's TSH level. (ii) To correlate iodine content of mother's urine with child's TSH level and also mother's milk content with child's TSH level. **Setting and Design:** Cross sectional study in human using consecutive sampling technique. **Materials and Methods:** Mother's urinary and milk iodine level was measured by Ammonium Persulfate Digestion Microplate method using Sandell-Kolthoff reaction in a sealing cassette (Hitachi, Japan) and child's TSH by ELISA commercial Kit from Eliscan (RFCL, India) based on classical sandwich technique. **Statistical Analysis:** Spearman's correlation was performed in quantitative variables. A p-value less than 0.05 and 0.001 were considered statistically significant and highly significant respectively. **Results:** The median mother's urine was 174.96 μg/L (97.39-215.43) and their respective median child TSH level was 3.86 mIU/L (2.66-4.80). Median mother's milk iodine was 129.90 μg/L (94.14-165.94).There was significant negative correlation between mothers' urinary iodine content and their child's TSH ($r2 = -0.391$, $p = 0.005$) and between mother's urinary iodine and their child's TSH ($r2 = -0.471$, $p = 0.001$). There was positive correlation between mother's milk iodine and mother urinary iodine but not statistically significant (($r2 = 0.261$, $p = 0.067$). **Conclusion:** Mothers urinary iodine, mother's milk iodine and child TSH are interrelated with each other.

Key words: Iodine, Milk Iodine concentration, Lactating, Correlation, Thyroid stimulating hormone

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INTRODUCTION

Iodine deficiency is a major public health problem throughout the world, particularly in developing countries. It affects the population of all age groups but pregnant women and children are the most vulnerable groups.[1] Iodine deficiency is still a significant public health problem in Nepal, 19.4% school age children has urinary iodine excretion <100 μg/L[2] which indicates mild iodine deficiency.[3] Breast fed infants are particularly sensitive to iodine deficiency because of the low iodine content of their thyroids and an extremely fast turnover of intra-thyroidal iodine.[4]

Iodine is considered unique among the trace elements in milk because it is avidly concentrated by the lactating breast. Breast fed infants are reliant on the iodine content of breast milk as it is the only natural source of iodine for them.[5] Iodine is required for the production of thyroid hormones, and is obtained solely from external sources. Thyroid hormones regulate various processes

Address for Correspondence:
Dr. Shruti Singh, Lecturer, Department of Biochemistry, KIST Medical College and Hospital, Imadol-6, Lalitpur, Nepal.
E-mail: shrutisngh@hotmail.com, **Tel:** +977-9851165178, **Fax:** +977-01-5201496.

of cellular metabolism, influencing all cells throughout life; particularly important is its role in normal brain development and cognition. It has been shown that insufficient iodine intake during pregnancy and lactation by the mothers may lead to irreversible brain damage in the newborns.[3,6,7] However, it is well-established that the iodine content of breast milk is critically influenced by the dietary intake of the pregnant and lactating mother.[8,9] Iodine requirements in pregnancy and during lactation are increased due to iodine transfer from the mother to the fetus/newborn infant, enhanced renal clearance during pregnancy and increased thyroid hormone synthesis in the pregnant woman.[10]

Iodine loss is more frequent in geographic regions where frequent topsoil erosion occurs and crops grown in such regions are more likely to be deficient in iodine, which directly influences the dietary intake of this essential micronutrient. The topography of Nepal leaves it under such a vulnerable situations.[11]

Nepal being a land locked country, the availability of iodine from sea food is restricted only to certain limited cities of Nepal. Most of the Nepalese population depends on local food productions for dietary supplements of iodine. Moreover mothers are deprived of nutrients due to several socioeconomic and cultural bondages or restrictions.[2]

Several indicators are used to assess the iodine status of a population: thyroid size, urinary iodine, and the blood constituents, thyroid stimulating hormone (TSH), and thyroglobulin (Tg).[12,13] However, all of these indicators have limitations. Since most of the iodine that is absorbed is excreted in the urine, the urinary iodine level is a good marker of a recent dietary intake, though not of thyroid function. Because 90% of the iodine absorbed by the body eventually appears in urine and therefore, is the index of choice for evaluating degree of iodine deficiency and its correction. Serum thyroid-stimulating hormone (TSH) is determined mainly by the level of circulating thyroid hormone, which in turn reflects iodine intake. TSH can be used as an indicator of iodine nutrition[14] in the newborn period,[15] and is used in many countries for routine newborn screening to detect congenital hypothyroidism.

Despite extensive studies on iodine content of school children's urine, mother's urine and child TSH; till date we didn't have much evidence of the iodine content in the milk of lactating mothers in the Nepalese population and its correlation with child TSH. Therefore, we have undertaken this study to evaluate the mother's urinary and milk iodine, child TSH and also correlation between mother's urinary iodine and mother's milk iodine with thyroid function status in the exclusively breast fed child.

MATERIALS AND METHODS

Setting

This Cross-sectional study was conducted in the department of Biochemistry in collaboration with the department of Gynaecology & Obstetrics and the department of Pediatrics and Adolescent medicine at B. P. Koirala Institute of Health Sciences (BPKIHS), Dharan, Nepal. A semi-structured proforma was designated for demographic data; dietary habits, and anthropological measurement.

Subjects

Mothers and their respective children attending the OPD of Department of Gynecology & Obstetrics and Department of Pediatrics and Adolescent medicine for routine checkup and/or immunization were selected for the study according to consecutive sampling technique. A total of 79 lactating mothers and their respective children, of age group 1-6 months, participated in this study where children were exclusively breast fed. Children or mothers having compromised medical conditions, or obtaining nutrition from sources other than breast milk and mothers taking iodine supplements or not giving consent to enroll themselves or their child in the study were excluded from the study. Ethical clearance was obtained from the Ethical committee on human Research of BPKIHS. Before accomplishing study, the study was explained to the women and informed consents were obtained. Maximum precaution was taken to reduce the pain and injury to the children in the course of study while not compromising the standard of the study.

Sample collection

Urine samples

Approximately 5 ml casual urine samples were collected from eligible mothers in tightly screw capped clean vials for measurement of urinary iodine. Urine sample was collected on the same day and close to the same time as that of milk.

Milk samples

Approximately 3 ml was collected from eligible mothers in tightly screw capped clean vials for measurement of milk iodine. Women collected breast milk either by manual expression or by breast pump.

Blood samples

Approximately 1 ml blood was collected from eligible children for measurement of TSH.

All were transported to the biochemistry laboratory.

Parameters

Demographic profile of mothers and their children were noted in the Proforma. Each child's height and weight were measured. A well calibrated weighing machine from KRUPS™/Infantometer was used to measure the weights in kilograms. For height measurement, mothers were asked to lay the baby straight on the table. A scale was used to mark the topmost point on the head and foot of the baby. Height in centimeters was immediately marked on the table with the help of measuring tape and noted in the proforma.

Measurement of urinary and milk iodine level were done by Ammonium Persulfate Digestion Microplate (APDM) Method using Sandell-Kolthoff reaction in a sealing cassette (Hitachi, Japan).[16,17]

Measurement of child serum TSH was done by ELISA commercial kit. The absorbance of calibrators and specimen was determined by using ELISA micro plate reader with a wavelength set at 450 nm. The concentration was evaluated by means of a calibration curve which was established from the calibrators supplied with the kit. The reference range of TSH in human serum is 0.3-6.16 mIU/L.

Reagent and chemicals

Ammonium persulphate (1.31mol/L), Arsenious acid solution (0.05mol/L), Sodium hydroxide (0.875 mol/L), Cerric ammonium sulphate (0.019 mol/L), Potassium iodate Calibrators: Stock A (1000mg/L), Stock B (10mg/L) and Stock C (100μg/L), Sodium chloride, Concentrated Sulphuric acid and Deionized Water. ELISCAN kit for TSH estimation manufactured by RFCL limited, India. All the reagents were prepared in deionized and double distilled water.

Seventy nine mothers/babies were involved in the study. Each milk samples were serially diluted with distilled water to determine linearity. All the undiluted milk samples showed precipitation. Each was diluted as follows: 1:2, 1:4, 1:8 and 1:16. Those samples were excluded from the study which showed precipitation even at 1:4, 1:8 and 1:16 dilution with distilled water. The mean recovery was 108.05% (Tables 1- 4).

Table 1: Showing concentration of sample of milk iodine at various dilutions

Dilution	Expected concentration (μg/l)	Observed concentration (μg/l)	% Expected
Undiluted		Precipitated	
1:2		Precipitated	
1:4		47.3025×4=189.21*	
1:8	189.21	20.17×8=161.36	85.28
1:16	189.21	18×16=290.29	153.92
Total			112.90

Table 2: Showing concentration of 2nd sample of milk iodine at various dilutions

Dilution	Expected concentration (μg/l)	Observed concentration (μg/l)	% Expected
Undiluted		Precipitated	
1:2		Precipitated	
1:4		38.687×4=234.75*	
1:8	234.75	23.90×8=191.25	81.47
1:16	234.75	18.79×16=300.79	128.13
Total			103.20

*Intra-assay: Within-run precision was determined by replicate determinations of three different milk samples in one assay using a dilution of 1:4

Table 3: Showing intra assay precision of milk sample

Milk sample	1	2	3
Number of replicates	20	20	20
Mean (Milk Iodine (μg/l)	128.04	189.2165	79.115
Standard deviation	16.269	13.298	7.730
Coefficient of variation (%)	8.5	10.38	9.77

*Inter-assay: Between-run precision was determined by replicate measurements of three different milk samples over a series of individually calibrated assays using a dilution of 1:4

Table 4: Showing inter assay precision of milk sample

Milk sample	1	2	3
Number of replicates	20	20	20
Mean (milk iodine (μg/l)	180.915	147.9495	87.4185
Standard deviation	37.67062	22.64026	21.43112
Coefficient of variation (%)	20.82	15.3	24.51

Standard graphs calibration curve

A calibration curve was prepared for each plate by plotting the logarithmic conversion of the means of absorbance at 405 nm (n = 2) on the y axis versus the iodine concentrations (0, 10, 25, 50, 100, 200, 300 and 400 μg/L iodine) on the x axis. The urine and milk iodine concentration was determined using linear regression. Water was used for the zero calibrator (Figure 1).

Statistical analysis

Data was entered in Microsoft Excel™ 2010 and then converted to IBM SPSS 17.0 for statistical analysis wherever required. Mean, Median with interquartile range and Standard deviation were calculated for descriptive statistics. Spearmen (rho) correlation analysis was used for correlation between mother's urinary iodine (MUI) and mother's milk iodine (MMI) and their respective child serum TSH. Initially data were checked for normal distribution by Kolmogorov-Smirnov test. Natural log transformation was used to transform the positively skewed data. Spearman's correlation was performed in quantitative variables between each other according to their distribution. A p-value less than 0.05 and 0.001 were considered statistically significant and highly significant respectively.

RESULTS

This study was undertaken to investigate mother's urinary iodine, an indicator of current iodine status in population, mother's milk iodine content and child's TSH level. We have used the mother's milk iodine as an indicator for intake of iodine for the child who is exclusively breast feeding. This study also investigated correlation of mother's urinary iodine excretion, mother's milk iodine content and child's TSH level.

Out of seventy nine mothers and children who participated in this study, only 50 samples each of mother and child were used for the analysis. Distribution of baby sex among the 50 samples was 56% male and 44% female (Figure 2).

Children involved in the study were 62%, 26% and 12% first child, second child and third child respectively of their mother (Figure 3).

In term of frequency of breast feeding in lactating mothers, 58% of mothers fed >15 times in 24hrs (Figure 4). Major sources (68%) of information regarding breast feeding were relatives or friends (Figure 5).

During breast feeding 56% mothers ate more non vegetarian food than before pregnancy. Similarly 44% ate more vegetarian diet since breast feeding. In addition, others dietary habits like alcohol intake, vitamins and mineral supplementation especially iodine supplementation, 10% mothers were found to have reduced consumption since breast feeding while 2% maintained the same intake and the majority (88%) responded that they never took these supplementations or alcohol.

On anthropometric measurement, majority of children had the mean height of 60.92±9.62 cm and mean weight was found to be 5.19±1.27 kg. The mean age of mothers participating in the study was 24.88±4.80 years and that of baby was 2.55±1.35 months (Table 5).

Iodine contents in mothers urine was estimated by Sandell Kolthoff method. The median mother's urine was found to be 174.96 μg/L (97.39-215.43). TSH level in children serum as estimated by ELISA (Sandwich Technique) had the median level of 3.86mIU/L (2.66-4.80). Correlation between child's TSH and mothers' urine was done by Spearman's rank correlation and result showed a negative correlation between mothers' urinary iodine content and child's TSH (Figure 6). The correlation coefficient was -0.391 with p value 0.005 which is statistically significant (Table 6).

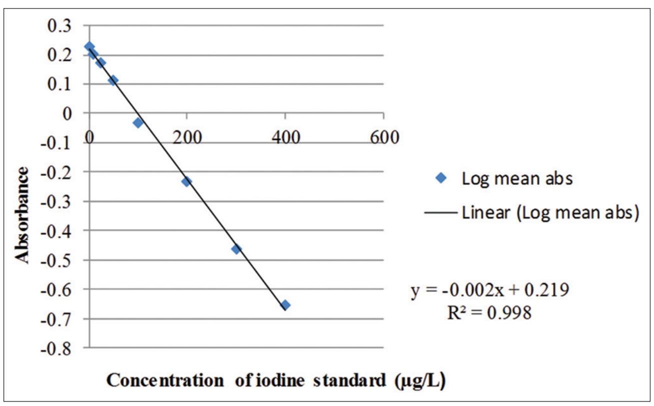

Figure 1: Standard curve of urine and milk iodine measurement by ammonium per sulphate Digestion micro plate (APDM) method)

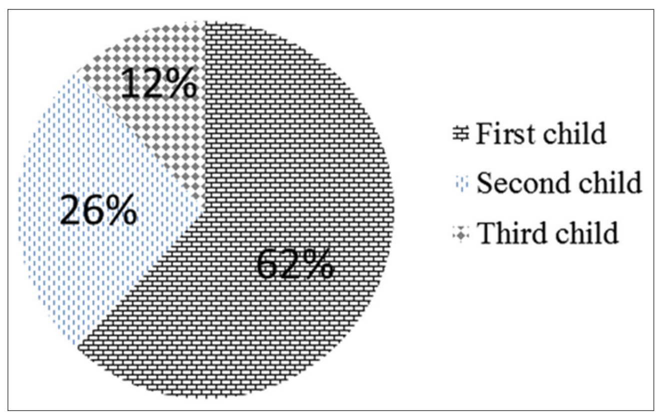

Figure 3: Distribution of Gravida

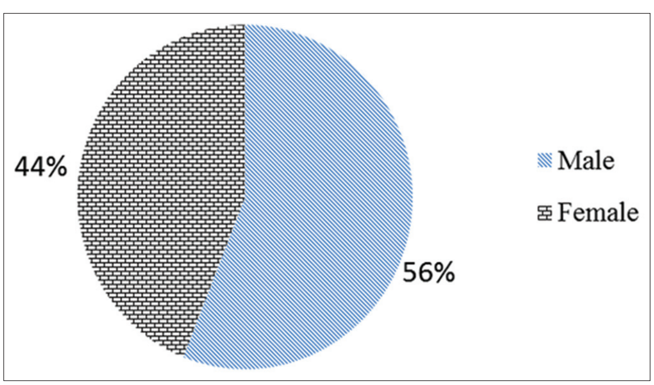

Figure 2: Genderwise distribution of children

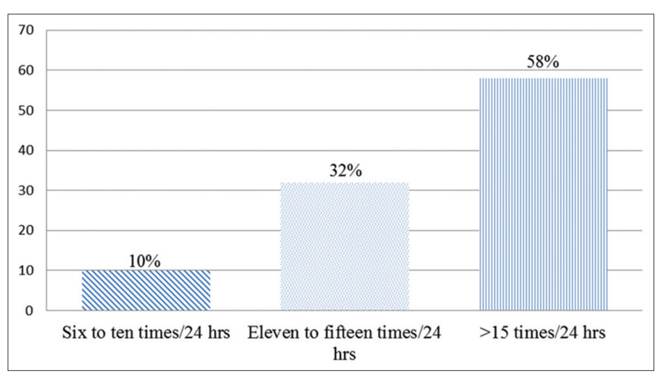

Figure 4: Pattern of Breast feeding by mothers to their children

Likewise Mother's milk iodine content was also estimated by Sandell Kolthoff method. The median mother's milk iodine was found to be 129.90 μg/L (94.14-165.94). Correlation between child's TSH and mothers' milk iodine was also done by Spearman's rank correlation test and result showed a negative correlation between mother's urinary iodine and child's TSH. The correlation coefficient was -0.471 with p value 0.001 which is statistically significant (Figure 7).

Similarly there was also positive correlation between mother's milk iodine and mother urinary iodine but it was not statistically significant. Correlation coefficient was 0.261 and p value 0.067 which is not statistically significant (Figure 8).

Twenty-six percent of lactating women showed insufficient iodine nutrition [MUI < 100 μg/L] based on median urinary iodine concentration whereas 74% showed adequate iodine nutrition [MUI ≥ 100 μg/L].

Twenty percent of child TSH values were above the threshold value of 5 mIU/L which suggests for moderate iodine deficiency in the population (Normal TSH < 3%). The exact cut off concentration of iodine in human milk has not been specified; however, values above 75 μg/L of milk may be considered as an index of sufficient iodine intake[18] and accordingly, 10% of the lactating mothers were found to have insufficient iodine intake.

Table 5: Anthropometric characteristics of mothers and children

Parameter	Mean	Median	SD
Baby's age	2.5508	2.365	1.35407
Mother's age	24.88	24.5	4.805
Baby's height in cm	60.92	60	9.625
Baby's weight in kg	5.19	5.1	1.273

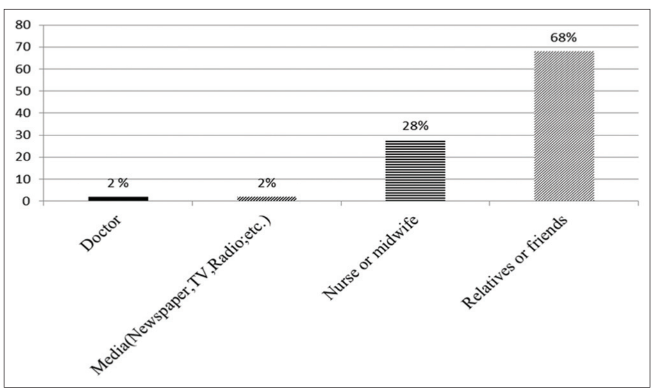

Figure 5: Source of information about breast feeding

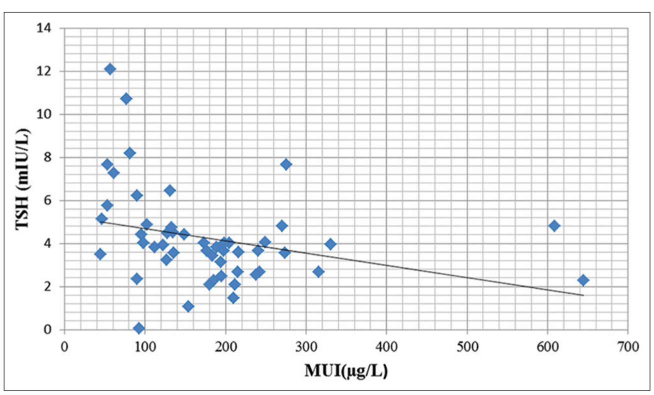

Figure 6: Scattered diagram showing correlation of mother's urinary iodine (MUI) and child TSH

DISCUSSION

We have estimated the iodine content in mother's urine and milk by Sandell-Kolthoff method[19] and child serum TSH by classical sandwich ELISA technique.[17,20,21]

The ammonium persulfate microplate digestion method was used because it is nonhazardous, nonexplosive, economical, soluble in water (making it easy to prepare

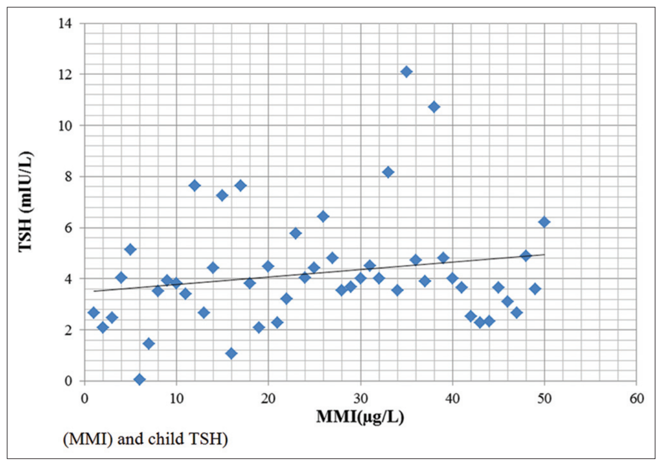

Figure 7: Scattered diagram showing correlation of mother's milk iodine (MMI) and child TSH)

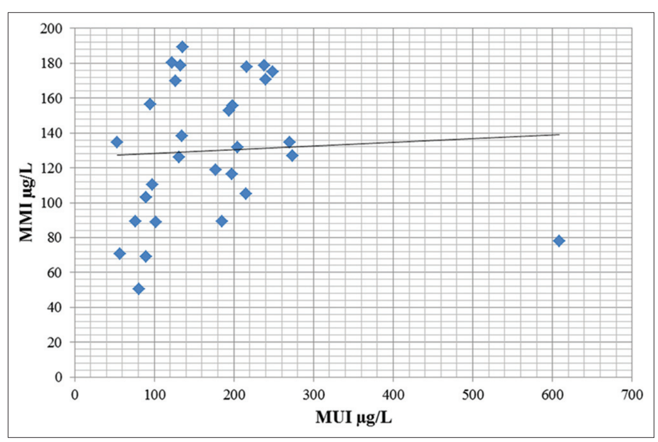

Figure 8: Scattered diagram showing correlation of mother's milk iodine (MMI) and mother's urinary iodine (MUI)

Table 6: Showing mother's milk iodine level, mother's urinary iodine level and child TSH level

Parameter	Mean	Median	SD	Percentiles 25	Percentiles 50	Percentiles 75
Iodine in mother urine (μg/L)	179.78	174.96	117.332	97.39	174.96	215.43
Iodine in mother milk (μg/L)	131.0964	129.9050	43.84005	94.1475	129.9050	165.9400
TSH (mIU/L)	4.2282	3.8650	2.21596	2.6600	3.8650	4.8050

concentrated solutions), a potent oxidizer, avoids ultraviolet irradiation, and eliminates the requirement of an exhaust fume hood. The present method includes ammonium persulfate as the oxidizing agent to eliminate the interfering substances in urine before the colorimetric measurement by the Sandell-Kolthoff reaction.[22]

This is probably the first study in Nepal which studied mothers' milk and urinary iodine for assessment of iodine nutrition, correlation of mothers' milk iodine and child's serum TSH level.Iodine deficiency (ID) has multiple adverse effects on growth and development due to inadequate thyroid hormone production.

The measurement of UI excretion can provide an accurate approximation of the very recent dietary intake of iodine[23] as about 90% of ingested iodine is excreted in the urine, and, therefore, is the index of choice for evaluating the degree of iodine deficiency and its correction.[24] But its determination provides little information on the long-term iodine status of an individual.[25]

In our study, MUI content was 174.96 μg/L(97.39-215.43) (Table 6) which reflects urinary iodine concentration in lactating mother was adequate. World Health Organization (WHO) states that the median UI concentration in a population should be greater than 100 μg/L, with less than 20% of the population excreting <50 μg/L.[26]

In this study, the proportion of the lactating mother with low median Urinary Iodine Excretion values below the cut-off value of 100 μg/L as given by WHO[26] was 26.0%, which is lower than the Nepal Micronutrient Status Survey in 1998 (35.1%) and the Nepal Iodine Deficiency Disorders Status Survey in 2005 (29.5%) while 74% of lactating mother showed adequate iodine nutrition [MUI ≥ 100 μg/L]. This may suggest that use of iodized salt and its accessibility in remote areas has improved the iodine status in the population.[27] In lactating women, the figures for median Urinary Iodine are lower than the iodine requirements because of the iodine excreted in breast milk.[14]

Mother milk iodine(MMI) was also estimated in this study and median MMI was 129.90 μg/L (94.14-165.94) (Table 6). Despite the importance of iodine to infant health, there are only few studies related to the iodine content of human milk in the scientific literature. These studies have shown that the mean iodine content of human milk is relatively low (9–32 μg/l) in women from areas with a high prevalence of goitre. However in areas where salt iodization programs have been implemented, iodine content of milk has increased.

The exact cut-off for concentration of iodine in human milk has not been specified; however, in one study,values above 75 μg/l of milk may be considered as an index of sufficient iodine intake.[28] In our study, median MMI of 129.90 μg/L may reflect sufficient iodine intake level in lactating mothers. According to the above cut off limit concentration, 90% of lactating mothers' population have showed sufficient iodine intake, although at national level, mothers milk iodine level has not been specified.

In one study, it has been mentioned that iodine content of breast milk varies widely due to maternal iodine intake. Mean breast milk iodine concentrations are reported as ranging from 5.4 to 2170 μg/L (median 62 μg/L) in worldwide studies.[29] Likewise in one more study,in conditions of iodine sufficiency, iodine content of breast milk was150–180 μg/L.[29,30]

Few studies have shown that breast milk iodine concentrations were higher in areas where iodized salt was consumed.[28] In another study, it has been shown that 2 years of supplementation of 10–20 μg/potassium iodide per kilogram with salt doubled the median iodine concentration in breast milk.[31] However in a study done in Saudi Arabia, mothers consuming iodized salt showed a significant increase in urine iodine, whereas the increase in milk iodine was not significant.[32]

Multicentric studies have shown that breast milk iodine concentrations vary in many countries with the same degree of iodine sufficiency or deficiency;[33] therefore environmental factors other than iodine depleted soil where crops are grown, may influence breast milk iodine concentration. Therefore, milk iodine level estimated in our study may or may not reflect iodine status of the lactating mothers in the general population distributed over wide and diverse geographical areas.

In our study, the mean child TSH value was found to be 4.22mIU/L (Table 6). According to WHO in areas of iodine deficiency more than 3% of neonatal whole-blood TSH values above the threshold of 5 mIU/L suggests iodine deficiency in the population.[34] TSH is an effective and sensitive biomarker for assessing changes in iodine status in newborns.[35-38] Elevated serum TSH in the neonate indicates insufficient supply of thyroid hormones to the developing brain, and therefore constitutes the only indicator that allows prediction of brain damage, which is the main complication of iodine deficiency. In our study, we have evaluated the TSH of exclusively breast feeding child. The frequency greater than 5 mIU/L was 20% which suggests moderate Iodine Deficiency.

One study has shown that, on a population basis, there is an inverse relationship between MUI and neonatal serum TSH levels. There was also evidence which support a link between a fall in maternal iodine intake and fetal thyroid dysfunction.[39] In our study, a strong negative correlation existed between MUI and child's TSH (p value is 0.005) (Figure 6). This result is in concordance with the previous study showing correlation between MUI and child's TSH. In one study, it was mentioned that there was no significant correlation between neonatal TSH and the MUI.[40,41] However several other studies have concluded that excessive level of urinary iodine corelates with higher neonatal TSH.They postulated that fetal thyroid in an area of mild iodine is more sensitive to the inhibitory effect of iodine.[42-44] Conversely in one study, a negative correlation was reported between MUI and child's TSH.[45]

In our study, there was signifiant negative correlation of MMI and child's TSH; p value 0.001 and correlation coeffiient was 0.471, which indicated that the infants' iodine status is affected by the mother's iodine status (Figure 7).

The dietary supply of iodine to the neonate and infant comes exclusively from breast milk in the first 6 months of life until they are shifted to complementary foods thereafter.[18] Correlation of MMI and child's TSH can be used for screening of iodine status of mothers as well as children.

Though in one study, higher breast milk iodine was correlated with a higher neonatal TSH[46] but in our study there is significant negative correlation of MMI and child's TSH. This may reflect a low iodine food consumption by lactating mothers which in part may be due to strategic location of Nepal falling within an endemic zone for iodine deficiency besides other socio-cultural and economic taboos prevailing in the society.[47] Iodine is also considered as the fastest acting antithyroid drug.[18] So, more than adequate or excessive iodine intake may lead to hypothyroidism.[48]

It is very noticeable that even if iodine has not been stored in the thyroid during fetal development, new born infant iodine requirement is fulfilled by breast milk. Iodine and thyroid hormones in breast milk are well-absorbed and may prevent impaired neurological development in infant.[28]

We have also analyzed the relationship between MUI and MMI. There was a positive correlation between MUI and MMI but not statistically significant (Figure 8). This is in agreement with a previous study done by wang et al.[49]

During pregnancy and lactation, the mammary glands iodide concentrating mechanism is responsible for adequate supply of iodine to the newborn.Concentration of iodine in human milk is 20–50 times higher than that of plasma.[28] This may be the reason for a less significant positive correlation between MUI and MMI in our study. However in one previous study, MUI and MMI levels were significantly correlated.[50]

The prediction of iodine intake is very difficult because amount of iodine in individual foods and water vary by a factor of 100.[51,52]

CONCLUSION

Our study has demonstrated that MUI is found to be adequate. Only 26% of lactating women showed insufficient iodine nutrition based on MUI. MMI level is also satisfactory which is very close to required iodine level in the milk. The MMI were comparable to other areas of the world that were considered moderately iodine deficient. Breast milk is the single source of iodine for breast fed infants in many countries during the critical period of brain development and the concentration of iodine in breast milk constitutes another index of iodine nutrition. Child's TSH values greater than 5 mIU/L was 20% suggesting moderate IDD among the children who participated in this study. There is a strong negative correlation between MMI and child's TSH, MUI and child's TSH which showed that infant thyroid status is based on mother's iodine intake through breast milk. Similarly there was also positive correlation between MMI and MUI but not statistically significant. This positive correlation may reflect the total intake of iodine by mother. These three parameters: MUI, MMI and child's TSH and their correlation among each other may be good biomarker for assessing iodine nutrition and identifying Iodine Deficiency Disorder (IDD) especially in neonates. These indicators can also be used for monitoring the success of an iodine supplementation program. More studies need to be done on a larger scale for assessment of the current status of iodine deficiency in Nepal and for supervision of proper iodine supplementation, which is required to prevent IDD with all its consequent morbidities.

Overall this study showed that Nepal is progressing towards sustainable elimination of IDD. But IDD still exists.

However, further similar studies from other parts of Nepal and elsewhere will be required to confirm our findings.

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Authors Contribution:
SS - Concept, literature search, collected and analyzed samples, data entry and statistical analysis, prepared the manuscript; **ML** - Concept and design of study, reviewed the literature and critical revision of the manuscript, helped in preparing the first draft of study; **NB** - Concept and design of study, reviewed the literature and critical revision of the manuscript; **NKB** - Reviewed the literature and critical revision of the manuscript. **DKU** - Reviewed the literature and critical revision of the manuscript.

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