Effect of iodine and iron status during pregnancy on maternal and neonatal thyroid functions: A prospective cohort study in Bope - Poddala health division

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ABSTRACT

Iodine and iron are the most common nutritional deficiencies in the world. It was reported that even mild iodine deficiency during pregnancy will affect maternal and subsequently neonatal thyroid functions and iron deficiency has multiple adverse effects on thyroid metabolism. Since iodine and iron deficiencies are common in pregnant women in Sri Lanka, its ultimate effect will be on the new born. The aim of the study was to assess the iodine and iron status in women during pregnancy and its effects on thyroid function of the mother and the newborn.

The study was carried out in the Bope-Poddala MOH division of Galle District. Four hundred and twenty-five pregnant women were enrolled and they were followed up during the course of the pregnancy until delivery. Maternal iodine and iron status was assessed using different parameters and its effect on babies was assessed by estimating neonatal urine iodine (UI) and neonatal thyroid stimulating hormone (nTSH).

The median maternal UI concentration of the sample was 175.2μg/L (IQR 106.3-263.4 μg/L),126.0μg/L (IQR 74.8 - 196.4μg/L),106.0μg/L (IQR 67.4-160.6 μg/L) in the first, second and third trimesters respectively indicating progressive reduction with the advancement of the pregnancy ($p = <0.001$). 41.7%mothers had insufficient UI concentration at the study entry and it was increased to 58.8% and 72.9% in the 2nd and 3rd trimesters. Median serum TSH in the 1st trimester, 1.3 mIU/mL (IQR 0.8 – 1.8 mIU/mL) was significantly increased ($p<0.001$) to 1.6 mIU/mL (IQR 1.2 – 2.1 mIU/mL) at the 3rd trimester. Median vales of fT4 for 1st and 3rd trimesters were 18.0 pmol/L and 15.5 pmol/L ($p=0.002$) respectively. Results confirmed poor iodine nutrition by UI during pregnancy and role of iodized salt in maintaining iodine nutrition throughout pregnancy was questionable. In contrast maternal thyroid status was maintained within reference range. Regarding salt iodine content >50% of brands did not contain iodine within the recommended range and this may be a contributing factor to the poor iodine nutrition seen among pregnant women. Only 10.9% of neonates had insufficient UI level (<100μg/L) and the median (IQR) UIC level was 105.20 (81.25; 142.00) μg/L indicating sufficient UI level. The median neonatal TSH level was 3.55 (2.50; 6.50) mIU/mL whereas 37.7% of neonates had neonatal TSH>5.0 mIU/mL indicating moderate iodine deficiency according to WHO criteria. Neonatal UI level had significant positive correlations with maternal 3rd trimester UI ($r=0.23$; $p<0.001$) but such a significant correlation was not observed between maternal UI and neonatal TSH. Prevalence of anaemia was low in early pregnancy (4.8%) but iron deficiency was significantly high (42.1% had ferritin < 15ng/mL). Iron status was significantly improved at the end of the pregnancy, most probably due to iron supplements. It was observed that maternal iron status had no significant effect on maternal as well as neonatal thyroid functions in this sample.

Although neonatal thyroid status was normal according to current reference values, it is worthwhile to assess long term effects of inadequate iodine status of mothers on the offspring. Iodine content of the salt products must be tightly regulated and manufacturing should be closely monitored.

This study was performed at the Department of Biochemistry and Nuclear Medicine Unit, Faculty of Medicine, University of Ruhuna, Sri Lanka. The results were published in three original papers in peer reviewed journals. In addition, seven abstracts were presented in national and international forums. The thesis was defended on 17th November 2015.

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Introduction

Iodine is a vital micronutrient required at all stages of life; foetal life and early childhood being the most critical phases of requirement [1]. Iodine deficiency during pregnancy and infancy may impair growth and neurodevelopment of the offspring and increase infant mortality [2]. Diet is the sole source of iodine, and it depends upon the iodine content of water and soil [3]. In many countries, iodized salt is the major source of iodine.

Pregnancy has a profound impact on the thyroid

gland and thyroid function and it is a stress for the thyroid gland. Production of thyroxine (T4) and triiodothyronine (T3) increases by 50%, along with a 50% increase in the daily iodine requirement [4].

Worldwide, about 39 million newborns are at risk of lowered intellectual capacity because of iodine deficiency, every year. It is important to note that maternal iodine deficiency is one of the major causes for children not to reach their full potential and on a list of modifiable biological and psychosocial risks encountered by young children; iodine deficiency was ranked in the third place [5].

The ongoing monitoring of population iodine status remains crucially important and particular attention may need to be paid to monitoring the status of vulnerable populations, such as pregnant women and infants [6]. Several indicators are used to assess the iodine status of apopulation: thyroid size by palpation and/or by ultrasonography, urinary iodine (UI) and the blood constituents, TSH and thyroglobulin [7].

According to a report published by the WHO in 2004 iodine intake in Sri Lanka was categorized as adequate and iodine nutrition as optimal [8]. It indicated that Sri Lanka has achieved a satisfactory control of iodine deficiency disorder (IDD) after the initiation of universal salt iodization programme in 1995. But mild to moderate iodine deficiency can still be there in a considerable percentage of population which was not screened.

However, a study done in Sri Lanka very recently revealed that median urinary iodine level in pregnant women was 113.7 µg/L, which is far below the WHO recommendation (between 150 and 249 µg/L), indicating inadequate iodine status of pregnant women in Sri Lanka and it was also found in 2010 that only 69.4% of salt at household level contained an adequate iodine concentration of >15 ppm in Sri Lanka [9]. These findings raised a question about the iodine nutrition status in Sri Lankan population at present, especially in pregnant women, after many years of salt iodization.

Iron deficiency (ID) is the most common nutritional deficiency state in the world, affecting more than two billion people globally (10). Iron deficiency can cause several adverse effects in various stages in life. It adversely affects the cognitive performance, behaviour, and physical growth of infants, preschool and school-aged children. Iron deficiency anaemia during pregnancy increases perinatal risks for mothers and neonates and increases overall infant mortality [11].

ID has multiple adverse effects on thyroid metabolism [12, 13]. It decreases circulating thyroid hormone concentrations, likely through impairment of the haem-dependent thyroid peroxidase (TPO) enzyme [14]. It is obvious that there is an association between iron status and thyroid hormone status. Since both iodine and iron deficiencies is common during pregnancy it are worthwhile to assess their effect on maternal and neonatal thyroid functions.

It has been shown that the consumption of iodized salt is not sufficient to maintain the optimal iodine level throughout the pregnancy even in some of the developed countries [15]. Therefore, it is important to assess the iodine level in each trimester to obtain the data of how universal salt iodization (USI) programme in Sri Lanka is successful in maintaining iodine nutrition throughout the pregnancy. The ultimate effect of maternal iodine and iron nutrition will be on the new born babies. Therefore, it is important to evaluate the thyroid function and iodine status of their new born babies by measuring blood spot TSH assay and urinary iodine excretion in newborn. This will finally give a clear idea of effectiveness of the iodized salt consumption in maintaining iodine nutrition during pregnancy and the effect of iron status during pregnancy on the thyroid function of the mother and the baby.

Methods

This study was conducted as a prospective cohort study in Bope- Poddala Health Division of the Galle District in the Southern Province. Required sample for this study was taken as 425 assuming that 50% of pregnant women were deficient in iodine with inflation of 10% to cover up possible termination of pregnancy during the study period and the dropouts.
All pregnant women with gestational age: ⩽ 12 week -s (as judged by the date of last menstrual period) who attended the five Maternal and Child Health (MCH) clinics in the Bope-Poddala MOH division were included. Pregnant women with previous history of thyroid and renal diseases were excluded.

Study was conducted in four phases. In the first phase of the study, basic details were obtained and size of the thyroid gland was assessed by palpation method and by ultra sound scanning. A spot urine sample was collected for urine iodine estimation. A blood sample was collected to assess thyroid function (thyroid stimulating hormone (TSH), free thyroxin (fT4) and serum thyroglobulin level), serum ferritin (SF) and haemoglobin (Hb) levels. Data collection was done by using an interviewer administered questionnaire as the tool. The pregnant women recruited for the study were followed at their respective clinics throughout the pregnancy period.

In the second phase, the subjects were met in their

2nd trimester and details about iron supplementation, information regarding gestational age at the time of second visit were obtained. A spot urine sample was collected again to assess the UI level in the second trimester.

In the third phase, the study subjects were visited again in their 3rd trimester and further details about iron supplementation, expected place of delivery and any change in contact details were obtained. A spot urine sample was collected to assess the UI level in the third trimester. Further, another blood sample was collected to assess same parameters as in the first phase.

In the fourth phase, details of the newborn were recorded. A heel prick blood sample and a urine sample were collected for the analysis of serum TSH and UI concentrations respectively.

Salt samples (both table salt and crystal salt) were collected from places where salt was being sold in the study area to determine the iodine content.

Urine iodine concentration was measured using ammonium persulfate method and the iodine content of iodated salt samples was measured using the iodometric titration method recommended by WHO/UNICEF/ ICCIDD (16) at the iodine contamination free laboratory of the Department of Biochemistry, Faculty of Medicine, Galle. To measure serum fT4, TSH and thyroglobulin immuno assay test kits (MP Biomedicals, Diagnostic Division, USA) were used. The Ferritin Immuno-assay Test Kits (MP Biomedicals, Diagnostic Division, USA) were used to estimate the serum ferritin concentrations and haemoglobin estimation was done by using automated haematology analyzer (Sysmex, USA).The DELFIA neonatal TSH kits (WasllacOy, Finland) were used to estimate the neonatal TSH concentrations.

Data were analyzed using SPSS version 17.0 for Windows. Descriptive data were presented as mean and standard deviation (SD) or median and interquartile range unless stated otherwise. Correlation and regression models were used to examine the associations between urinary iodine and other variables recorded as continuous numerical variables. When comparing different groups, student t-test (for two groups), and analysis of variance (for three or more groups) was used to detect differences in numerical variables and Chi square test was used to detect differences in categorical variables. Details of the statistical calculations used for individual analysis are given in the relevant results sections. Two-tailed p value less than 0.05 was selected as the level of statistical significance.

Ethical approval for the study was obtained from the Ethical Review Committee of the Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka and informed written consent was obtained from each of the participants who were selected for the study.

Results

Iodine content of iodized salt

There were 89 salt samples of salt comprising 30 of crystal and 59 of powdered salt (table salt) belonged to 42 different brands (Table 1). In the total sample, there were 15 brands of crystal salt and 27 of powdered forms.

The overall median iodide level of the total sample was 20.41 ppm (range 0.0 to 73.81) where as the crystal salt had a median of 17.77 ppm (range 3.70 to 73.81) and powdered salt had 21.15ppm (range 0.0 to 41.24). Although the median iodine level was found within the specified range (15-30 ppm) our analysis revealed that 23.6% of samples (11 crystal and 10 powdered salt), had iodide levels below 15ppm and one powdered salt sample did not even contain a detectable amount. On the other hand 12.4% samples (4 crystal and 7 powdered) had iodine level above the upper limit of the recommendation.

Maternal iodine status

The adequacy of UI levels in pregnancy was analyzed using the criteria given by WHO/UNICEF/ICCIDD, 2007 and tabulated in Table 3. It has shown that 177 (41.7%) mothers had insufficient UI concentration at the study entry. During the 2nd trimester, 204 (58.8%) were detected as having insufficient UI levels and at the 3rd trimester, 272 (72.9%) pregnant women had insufficient UI levels.

According to palpation method 84.1% (n= 354) subjects did not have a palpable or visible goitre. Fifty five (13.1%) study subjects had a goitre that was palpable but not visible. Only 2.9% (n =12) had goitres which were not diagnosed previously.

The median thyroid volume was 5.16 mL (IQR 4.30; 6.10 mL) as measured by US scanning. The thyroid volume has a significant direct relationship ($p<0.001$) with the gland size as it increases steadily with the classification of goitre.

It was revealed that younger the mother (Age < 25 years) the thyroid volume is significantly ($p=0.04$) lower than the older mothers and mothers of their first pregnancy had significantly lower ($p<0.001$) thyroid volume than those were pregnant for the 2 time or more.

Table 1: Characteristics of salt usage & the type[1]

Characteristics	Salt type		Significance[2]
	Crystal (n=30)	Powder (n=59)	(χ^2 - value)
Place of purchase			
Retail shops	20 (22.5)	37 (41.6)	0.52; p=0.85
Supermarkets	7 (7.9)	17 (19.1)	
Weekly fair	3 (3.4)	5 (5.6)	
Exposure to sunlight			
Yes	7 (7.9)	10 (11.2)	0.63; p=0.47
No	23 (25.8)	49 (55.1)	
Shelf life			
<365 days	22 (24.7)	53 (59.6)	4.08; p=0.04
=365 days	8 (9.0)	6 (6.7)	
Period of storage			
<90 days	18 (20.2)	34 (38.2)	0.05; p=0.83
=90 days	12 (13.5)	25 (28.1)	
Place of storage			
Shelf	15 (16.9)	33 (37.2)	0.28; p=60
Floor	15 (16.9)	26 (29.1)	

[1]Results presented as median (inter-quartile-range)
[2]Chi-square test at 2 df is 94.3; p = <0.001

Table 3: Adequacy of urinary iodine excretion level during pregnancy[1]

Iodine Status[2]	1st Trimester	2nd Trimester	3rd Trimester
Excessive (=500 µg/L)	4 (0.9)		2 (0.5)
Above requirements (250 -499 µg/L)	120 (28.2)	37 (10.7)	24 (6.4)
Iodine Sufficient (150 -249 µg/L)	124 (29.2)	106 (30.5)	75 (20.1)
Mild Iodine Deficiency (<150 µg/L)	159 (37.4)	168 (48.4)	232 (62.2)
Moderate Iodine Deficiency (<20 -49 µg/L)	16 (3.8)	32 (9.2)	39 (10.5)
Severe Iodine Deficiency (<20 µg/L)	2 (0.5)	4 (1.2)	1 (0.3)
Total	**425**	**347**	**373**

[1]Results presented as n (%)
[2]Iodine status (urinary iodine levels) was given by WHO/UNICEF/ICCIDD, 2007; "excessive" means in excess of the amount required to prevent and control iodine deficiency.

In this study the thyroid function tests were done to assess the iodine nutrition of subjects at the study entry (1st trimester) and at the end of the 3rd trimester. The Median serum TSH level of the subjects in their 1st trimester was 1.3mIU/mL (IQR 0.8 – 1.8 mIU/mL) and at the end of the 3rd trimester it was significantly increased ($p<0.001$) to 1.6 mIU/mL (IQR 1.2 – 2.1 mIU/mL). Median vales of fT4 for 1st and 3rd trimesters were 18.0 pmol/L and 15.5 pmol/L ($p=0.002$) respectively. There were no subjects with fT4 values below the lower limit (<6.4pmol/L) of the reference (hypothyroid) range in both first and third trimesters.

A statistically significant association between UI levels and free T4 levels in the third trimester ($p=0.04$) was observed. The pregnant women with thyroid size of grade 0 (n=354) had higher fT4 (mean levels of 19.23 pmol/L) than the mothers of grade 2 (n= 12; mean level of 18.13 pmol/L). However, the difference observed was not statistically significant ($p=0.74$)

Maternal iron status

The mean Hb level at the study entry was 12.4 ± 0.92 g/dL and it dropped to 12.1 ± 1.1 g/dL by the third trimester (z test =4.47; $p<0.001$). The median SF level was 17.5 ng/mL (IQR 9.2; 30.0 µg/L) at the study entry and it improved to 30.3 ng/mL (17.4; 50.8 µg/L) at the third trimester (z test = 9.2; $p<0.001$).

It was noted that only 20 (4.8%) mothers were anaemic (Hb<11.0 g/dL) in the first trimester. However, the number of anaemic mothers increased to 49 (13.8%) at the end of the third trimester ($p<0.001$). In contrast, 178 (42.1%) pregnant women were iron deficient (SF <15.0 ng/mL) in the first trimester, but iron deficiency has been significantly ($p<0.001$) reduced as only 64 (17.5 %) pregnant women were having SF <15.0 ng/mL at third trimester (Table 4).

Table 4: Frequency distribution of Hb level and serum ferritin level

Characteristics	Trimester				Significance
	1st		3rd		
	n	%	n	%	
Haemoglobin Level (g/dL)					
Low (<11.0)	20	4.8	49	13.8	$\chi^2 = 19.13$
Normal (=11.01)	396	95.2	305	86.2	df=1, p<0.0 01
Serum Ferritin Level (ng/mL)					
Low (<15.0)	178	42.1	64	17.5	$\chi^2 = 55.82$
Normal (=15.01	245	57.9	302	82.5	df=1, p<0.001

In this study it was noted that 181 mothers (51.1%) obtained iron tablets (FeSO4) from antenatal clinics and the remainder (n=171, 48.3%) consumed commercial preparations of iron tablet/capsules (Ferrous fumarate, gluconate, etc.) purchased from the private sector.

Of the 64 pregnant women who were iron deficient (SF<15.0 g/dL) at the third trimester, 31 (48.4%) obtained the iron supplements from Government antenatal clinics and the rest (n=33; 51.6 %) from the private sector. However there was no significant difference (p=0.92) in the number (%) of subjects with iron deficiency observed in the two categories.

The total content of iron consumed by each woman was calculated during pregnancy by the amount of elemental iron in each preparation and the number of days consumed. The pregnant women who received iron supplement from government clinics had a median intake of 10,080.0 mg (IQR 8,820.0; 10,500.0 mg) whereas those who purchased from the private sector had median intake of 8,400.0 mg (7,896.0; 8,750.0 mg). A statistically significant difference is seen in the two intakes (z test 1.94; p = 0.05).

The correlation coefficients on parameters at each investigation points are described in Table 5 (at the study entry) and Table 6 (at the third trimester). A significant negative correlation (r=-0.18; p<0.001) was seen between maternal serum TSH and fT4 levels and significant positive correlation (r=0.12; p=0.01) between serum ferritin and Hb levels at the study entry. However, it was evident that in addition to these correlations there were significant negative correlations also (Table 6) between serum TSH and Hb (r=-0.14; p=0.01) and serum Ferritin with fT4 (r= -0.19; p=0.01). No significant correlation between Tg and anaemia or iron status was seen in the first trimester.

Table 5: Correlation of the thyroid function test, anaemia and iron status in first trimester

	TSH	fT4	Hb	Ferritin	Tg
TSH	1	-0.18 (<0.001)	0.01 (0.79)	0.01(0.93)	0.05 (0.54)
fT4	-0.18 (<0.001)	1	-0.02 (0.71)	0.07 (0.16)	-0.01 (0.92)
Hb	0.01 (0.79)	-0.02 (0.71)	1	0.12 (0.01)	-0.06 (0.47)
Ferritin	0.01 (0.93)	0.07 (0.16)	0.12 (0.01)	1	-0.06 (0.52)
Tg	0.05 (0.54)	-0.01 (0.92)	-0.06 (0.47)	-0.06 (0.52)	1

Table 6: Correlation of the thyroid function test, anaemia and iron status in third trimester

	TSH	fT4	Hb	Ferritin
TSH	1	-0.11 (0.03)	-0.14 (0.01)	0.01 (0.89)
fT4	-0.11 (0.03)	1	0.05 (0.36)	-0.19 (0.01)
Hb	-0.14 (0.01)	0.05 (0.36)	1	0.12 (0.02)
Ferritin	0.01 (0.89)	-0.19 (0.01)	0.12 (0.02)	1

Effect of maternal iodine and iron status on neonatal thyroid function

The median (IQR) neonatal TSH (nTSH) level was 3.55 (2.50; 6.50) mIU/L whereas the median (IQR) UIC level was 105.20 (81.25; 142.00) µg/L. There were 4 infants with nTSH above the cut-off value of 20.00 mIU/L with the highest value of 39.00 mIU/L. However, all had normal serum TSH and fT4 levels. Therefore, none of these babies were confirmed as having congenital hypothyroidism. There were 216 (62.3%) babies with nTSH<5.0 mIU/L and 37.7% of neonates had nTSH>5.0 mIU/L. Further, 285 (89.1%) had normal urinary iodine levels of ≥100 µg/L. Only 35 babies (10.9%) had insufficient (<100 µg/L) urine iodine levels.

A new born who has a birth weight of less than 2500g was considered as low birth weight infant (16 WHO, 2011a). The mean ±SD birth weight of the study sample was 3.01±0.49 Kg. The comparison of neonatal data with sex of the neonate is presented in Table 7. It was revealed that the mean ±SD birth weight of males (3.10±0.46 kg) was significantly higher when compared to that of female counterparts (2.89±0.50kg; t-test 4.09; p<0.001). The median urinary iodine level in this sample was 105.5 (IQR 81.20; 142.0). The mean ±SD urinary iodine level among males (208.84±89.20µg/L) was significantly higher when compared with females babies (188.74±80.80µg/L; t-test 2.08; p=0.04).

The correlations between the measured parameters of the neonates with the maternal thyroid (serum TSH, fT4, urinary iodine level and thyroid volume) and iron status (Hb and serum ferritin) were analysed and the results are tabulated in Table 8. The birth weight of infants showed a significant positive correlation (r=0.13; p=0.01) with mother's thyroid volume. Neonatal blood spot TSH level showed significant negative correlations with mother's 3rd trimester fT4 level (r=-0.10; p=0.04) and Hb (r=-0.10; p=0.03) whereas the negative correlation with serum TSH (r=-0.01) did not reach to a significant level (p=0.80). Further, neonatal urinary iodine level had significant positive correlations with mother's 3rd trimester urinary iodine (r=0.23; p<0.001) and fT4 (r=0.14; p=0.01) levels.

Table 7: General characteristics of the baby's in the study sample

Parameter	Unit	males	females	t-test	p-value
n		191	171		
Birth w eight	kg	3.10±0.46	2.89±0.50	4.09	<0.001
Urinary iodine	µg/L	208.84±89.20	188.74±80.80	2.08	0.04
nTS H	mIU/L	5.20±3.60	5.39±4.60	-0.44	0.66

Table 8: Correlation between neonatal and maternal thyroid and iron parameters

		Baby's			Mother at 3rd trimester				
		BW	nTSH	nUI	UI	TSH	TV	Hb	SF	fT4
Baby's	BW		0.04	0.04	0.06	-0.01	0.13	0.02	-0.03	-0.02
			(0.45)	(0.47)	(0.27)	(0.94)	(0.01)	(0.68)	(0.57)	(0.72)
	nTSH	0.04		0.03	0.03	-0.01	-0.02	-0.10	-0.06	-0.10
		(0.45)		(0.60)	(0.55)	(0.80)	(0.75)	(0.03)	(0.30)	(0.04)
	nUI	0.04	0.03		0.23	0.02	-0.01	-0.03	-0.01	0.14
		(0.47)	(0.60)		(<0.001)	(0.79)	(0.87)	(0.62)	(0.92)	(0.01)
Mother at 3rd trimester	UI	0.06	0.03	0.23		-0.01	0.10	-0.01	0.01	0.01
		(0.27)	(0.55)	(<0.001)		(0.81)	(0.03)	(0.88)	(0.83)	(0.98)
	TSH	-0.01	-0.01	0.02	-0.01		-0.05	-0.01	0.01	-0.08
		(0.94)	(0.80)	(0.79)	(0.81)		(0.36)	(0.95)	(0.92)	(0.13)
	TV	0.13	-0.02	-0.01	0.10	-0.05		0.02	0.11	0.06
		(0.01)	(0.75)	(0.87)	(0.03)	(0.36)		(0.78)	(0.04)	(0.23)
	Hb	0.02	-0.10	-0.03	-0.01	-0.01	0.02		0.32	-0.01
		(0.68)	(0.03)	(0.62)	(0.88)	(0.95)	(0.78)		(<0.001)	(0.84)
	SF	-0.03	-0.06	-0.01	0.01	0.01	0.11	0.32		-0.14
		(0.57)	(0.30)	(0.92)	(0.83)	(0.92)	(0.04)	(<0.001)		(0.01)
	fT_4	-0.02	-0.10	0.14	0.01	-0.08	0.06	-0.01	-0.14	
		(0.72)	(0.04)	(0.01)	(0.98)	(0.13)	(0.23)	(0.84)	(0.01)	

Discussion

Iodized salt is the main source of iodine in Sri Lankan population and it has been the main strategy to control the Iodine Deficiency Disorder (IDD). It was reported that even in iodine sufficient or mildly iodine deficient (MID) areas, iodine deficiency during pregnancy frequently appears and thyroid gland cannot meet the demand for increasing the production of thyroid hormones. Its effect may be damaging the neurodevelopment of the foetus. Therefore, it is important to prevent even mild iodine deficiency during pregnancy to have a better pregnancy outcome. Investigations on iodine nutrition in pregnancy in Sri Lanka were very few. No national level studies have been conducted to assess the iodine nutrition in pregnant women in Sri Lanka up to 2010. The study carried out by Medical Research Institute (MRI) in 2010 revealed that the overall median urine iodine concentration among pregnant women was 113.7µg/L indicating an iodine deficiency in Sri Lankan pregnant women (9) and it was not a follow up study. The present study analyzed the maternal iodine status prospectively in

detail using thyroid size, thyroid profile and UI level. In the present study it was clearly evident that the median UI concentration reduced significantly during the course of pregnancy ($p< 0.001$). This progressive decrease in urine iodine concentration can be attributed to the increased demand of iodine as a result of advancement of the pregnancy [17]. Indeed, the apparent better iodine status in early pregnancy may be due to the increase of glomerular filtration during first trimester causing an increased UIC and, therefore an increased loss of iodine from the body. The reduction in urinary iodine level with the advancement of gestational age in this study is quite compatible with the results of studies done in Bangladesh [18], Congo [19] and Nigeria [20]. In contrast results of national health and nutrition examination survey (NHANES) in USA showed that UI levels were increased in the second and third trimesters when compared to the first trimester [21]. This increase in iodine status was attributed to the usage of supplements containing iodine during pregnancy.

Present study showed that only 64% of salt products contained iodine within the recommended range and a previous investigation too [9] showed that household usage of adequately iodized salt was 69.4%. These factors may also be contributing to the poor iodine nutrition status evident during pregnancy in the study population and it suggests that the consumption of iodized salt has not met the increased demand for iodine during pregnancy in this study population.

The increase in serum TSH level and decrease in fT4 level with the advancement of pregnancy in our study was compatible with the findings of the study done in Bangladesh [18]. The UI by the TSH levels in both first and third trimesters, showed that vast majority of the subjects had TSH levels within normal range irrespective of the UIC in both first and third trimesters. Even though UIC decreased towards the end of the pregnancy serum TSH levels were maintained within the reference range.

The median fT4 level was significantly reduced from18.0pmol/L (IQR 14.2-21.9) in the first trimester to15.5 pmol/L (IQR 11.6 - 21.9) towards the end of the pregnancy ($p=0.002$). Even though fT4was not a good indicator of iodine nutrition during pregnancy, normal levels indicated that the thyroid function was maintained properly in the study sample. There was an association between thyroid size and mean fT4 levels: women with thyroid size of grade 0 ($n=351$) had higher fT4 (mean levels of 19.23pmol/L) than the women of grade 2 ($n= 12$; mean level of 18.13pmol/L). However, this observed difference was not statistically significant ($p=0.74$). Other reasons for goitre (immune markers) were not evaluated in this study.

Even though iodine nutrition among the study population was poor based on the UI status, the results of the thyroid function tests appears otherwise. Urine iodine levels in the first and third trimesters did not show any significant association with thyroid function (TSH and fT4) tests. One of the most probable reasons may be not using the reference ranges for the thyroid function test specific to pregnancy, and there must be a concrete agreement regarding the method and the reference ranges to be used in the assessment of thyroid functions during pregnancy in our population.

Studies in Sri Lanka have indicated different prevalence rates for anaemia during pregnancy. It was common to see from findings of many of the studies that prevalence rates of anaemia during pregnancy was relatively low when compared to the national figures [22,23]. The present study also showed low prevalence rate (4.8%) in contrast to the figure of 16.7% given in the most recent national survey [24].

The usual trend that was observed in relation to the iron stores in pregnant women was the depletion of iron stores towards the end of the pregnancy [25, 26, 27]. Senanayake et al., in 2010 (28) showed a similar result in a study done in Sri Lanka. In contrast to these studies a significant improvement in ferritin level seen in this study sample illustrates that the iron stores of the overall sample has been improved towards the end of the pregnancy even though the prevalence of anaemia was increased. It may be due to the successful iron supplementation programme carried out in Sri Lanka at present.

It was observed that almost equal percentage of pregnant women received iron supplements either from government or private sectors. Further, there was no difference in the proportion of anaemia and iron deficiency observed in the two groups. The commercial preparations are much more expensive than the FeSO4 given in the government sector. The results showed that almost half of the pregnant women in this sample preferred commercial preparations obtained at a very high cost despite the fact that iron supplements are freely available at the antenatal clinics conducted by the Health Department. The most probable reason for that may be the common perception among some medical personnel and the pregnant women that commercial iron preparations are better in improving iron status and it has been proven wrong by this study.

It has been suggested that those who are iodine deficient may also be iron deficient and if IDA is a nutritional factor that influences the pathogenesis of IDD, it may have a greater impact on IDD than goitrogens because of its high prevalence in vulnerable groups [29]. The requirement for thyroid

hormone during pregnancy sharply increases [30] and it is obvious that concurrent iron deficiency may further impair the maternal thyroid function in iodine deficient pregnant women.

Assessing urine iodine level as a measure of iodine nutrition in new born is a very difficult task and it has not been done in Sri Lanka. The main challenge in measuring urine iodine is the difficulty in collecting urine samples from the neonate [31].

The study done at Turkey showed that 10.3% of the newborns and 56.8% of mothers were iodine deficient [32]. A recent study in Iran revealed that prevalence of neonatal iodine deficiency was 14.2% and the urine iodine level was < 100 µg/L in 33.9% of mothers [33]. A common finding in these studies including the present study is the lower prevalence of iodine deficiency among neonates despite the higher prevalence of maternal iodine deficiency. Urine iodine level among neonates in the present study had a significant positive correlation with mother's 3rd trimester urinary iodine ($r=0.23$; $p<0.001$) similar to the Iranian study ($r=0.46$, $P<0.001$) [33].

The results of the present study showed that 37.7% (n=131) of neonates had TSH level >5.0 mIU/L. It has been shown in Thailand that 8.9% of neonates had TSH >5.0mIU/L [34]. A neonatal TSH frequency of >5.0mIU/L has been reported in less than 3% of neonates in several mildly iodine deficient regions suggesting that neonatal TSH may not be sensitive enough to evaluate iodine status when there was mild iodine deficiency. A study from Australia using a sensitive TSH assay found that only 2.2% of neonates had a TSH value >5.0% despite a median UIC of 85.0 µg/L among pregnant women [35].

When assessing the iodine nutritional status, it has been proposed that neonatal thyroid-stimulating hormone concentration is a good indicator of iodine deficiency in the population. The WHO has proposed to use the results of screening programmes for congenital hypothyroidism in neonates as an additional index for the evaluation of iodine status of the population. A frequency of neonatal TSH concentrations > 5.0mIU/L below 3% was proposed as indicating iodine sufficiency [36]. In mild iodine deficiency the frequency may be 3.0–19.9%. The frequencies of 20.0–39.9% and above 40% may be found in moderate and severe iodine deficiency respectively The iodine deficiency in the present study was moderate according to WHO criteria [36] that the median (IQR) neonatal TSH level was 3.55 (2.50; 6.50) mIU/L. These findings are similar to the data from elsewhere [37]. A study done in Thailand showed that the mean neonatal TSH was 2.40 (SD 1.56) mIU/mL and according to them, although the median neonatal TSH concentrations were within normal ranges, the proportion with TSH >5 mIU/L ranged from 6.0- 14.0%, an indication of iodine insufficiency in both mothers and fetuses during pregnancy [34].

In the present study we studied the correlation of neonatal TSH with maternal TSH and fT4 level levels. The blood spot TSH level showed significant negative correlations with mother's 3rd trimester fT4 level ($r=-0.10$; $p=0.04$) whereas the negative correlation with serum TSH ($r=-0.01$; $p=0.80$) did not reach a significant level. A recent study also showed that neonatal TSH is not associated with maternal thyroid function in either of the two trimesters studied [38].

There were evidences to suggest that iron deficiency affect the thyroid functions [39, 40] and therefore the effect of maternal iron status on neonatal thyroid function was assessed in the present study. According to the results, there was a negative correlation between neonatal TSH and third trimester serum ferritin level ($r=-0.16$; $p=0.30$) though not significant, but the blood spot nTSH level showed a significant negative correlation with mother's 3rd trimester Hb level ($r=-0.10$; $p=0.03$). The clinical relevance of this significant negative correlation is not clear but it indicated that maternal iron status does not influence the neonatal thyroid function.

Conclusions

Although iodization of salt is compulsory by law in Sri Lanka it appears to be not well monitored as significant number of salt products do not contain recommended levels of iodine. A proper monitoring system at the production level to assess iodine concentration appears important.

About three fourth of pregnant women were iodine deficient in the third trimester and out of that, the majority had mild iodine deficiency. This finding quite agrees with the recent findings of iodine nutrition of pregnant women in Sri Lanka. The effectiveness of iodized salt in maintaining iodine nutrition during pregnancy is questionable and it suggests that iodized salt consumption is not meeting the increased demand for iodine.

The overall maternal iron status was satisfactory. Effect of maternal iron status on maternal thyroid function was not significant. Higher prevalence of iron deficiency in early pregnancy should be addressed.

Prevalence of iodine deficiency among newborn in this sample is relatively low (10.9%) in contrast to maternal iodine deficiency. Neonatal UI level indicated significant positive correlation with third trimester maternal UI level and maternal fT4 but the

correlation between neonatal TSH and maternal UI level was not significant in this study. Higher prevalence of neonates with TSH >5 mIU/L (37.7%) indicates moderate iodine deficiency during pregnancy and in neonates in this study sample but interpretation should be done carefully. Maternal iron status does not influence the neonatal thyroid function in this study sample.

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