Thyroid Functions in Bamboo-Shoots Consuming Children of Tripura, North-east India

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Abstract

Purpose

Information on thyroid functions in populations consuming potent antithyroidal bamboo-shoots (BS) was found scanty. Therefore, to assess thyroid function in BS consuming children was found relevant.

Methods

This cross-sectional study included 127 children from 3 villages. Thyroid volume (TV), free thyroxine (FT_4), free triiodothyronine (FT_3), thyroid stimulating hormone (TSH), thyroglobulin antibody (Tg-Ab), thyroid peroxidase antibody (TPO-Ab), urinary iodine (UI), urinary thiocyanate (USCN), salt iodine (SI), water iodine (WI), and BS consumption pattern were assessed.

Results

Variable-wise overall mean±SDs/medians/interquartile ranges (IQR) were - age: 8.69±1.69/8.77/7.22-9.74 y, TVol: 0.82±0.27/0.82/0.67-0.96 ml, FT_4: 19.5+5.6/20.1/16.9-22.0 pmol/L, FT_3: 4.27+1.24/4.22/3.35-4.96 pmol/L, TSH: 2.44+1.39/2.33/1.60-3.15 mIU/L, Tg-Ab: 15.2+10.0/12.5/11.1-14.5 IU/ml, TPO-Ab: 5.58+12.25/3.89/2.90-5.11 IU/ml, UI: 117.4±58.5/108/73-160.5 µg/L, USCN: 0.99+0.67/0.8/0.5-1.2 mg/dl, WI: 4 .69±4.56/3.0/1.35–7µg/L, and BS consumption: 153.8±01.4/119/71.4–214.3 g/person/day respectively. Only 80.3% salt samples had ≥15 ppm iodine. Thyroid dysfunction prevalence was 6.3%. There were positive correlations between TV and FT_4 (r=0.2466, *p*=0.005), UI and TSH (r=0.2633, *p*=0.003), TSH and FT_4 (r=0.2135, *p*=0.016), TSH and FT_3 (r=0.1898, *p*=0.033), USCN and FT_4 (r=0.2477, *p*=0.005), Tg-Ab and TPO-Ab (r=0.3768, *p*=<0.001), and negative correlations between Tg-Ab and TSH (r=-0.2024, *p*=0.023), Tg-Ab and FT_4 (r=-0.1869, *p*=0.035). In boys, USCN had a positive correlation with TPO-Ab (r=0.3069, *p*=0.018). The village having highest levels of BS consumption (*p*=0.037) and median UI showed higher TSH levels (*p*=0.037) and higher FT_3 level (*p*=0.001) compared to the village of lowest BS consumption.

Conclusions

The BS consuming children appear at risk of developing hypothyroidism by Tg-Ab, which may partly be diminished by reducing BS consumption.

Introduction

- Thyroid gland synthesizes thyroid hormones (TH) – thyroxine (T_4) and triiodothyronine (T_3) which play pivotal roles in growth, development and functions of body organs. For the synthesis of TH, iodine is essential in trace amount [1, 2]. However, about 80% of T_3 is formed from extrathyroidal deiodination of T_4 [1]. As FT_4 and FT_3 (unbound forms) are accessible to peripheral tissues and regulate biological activities of T_4 and T_3 respectively, FT_4 and FT_3 are considered clinically most relevant for evaluating thyroid disorders [3]. Circulating levels of T_4, T_3 and TSH remain relatively constant and reflect their 'set points' of hypothalamic–pituitary–thyroid (HPT) axis [4]. For each hormone, every individual has a HPT axis set point showing circadian rhythm determined by genetic makeup [5] and influenced by factors like age [6], iodine intake [7], seasons [8], smoking [9], antithyroid substances and nutrient deficiencies [1]. Thyroid stimulating hormone stimulates TH synthesis and release, increasing Na^+/I^- symporter (NIS), thyroglobulin and thyroid peroxidase synthesis, iodine organification, and thyroglobulin turnover [10].

Natural sources of iodine are iodides of food and water [1]. An individual having a UI of 100–199 µg/L is considered to have adequate iodine nutrition, indicating an iodine intake of 150-<300 µg/day [11]. Individuals consuming foodstuffs deficient in iodine and/or rich in goitrogens may face iodine deficiency (ID) [1, 2, 12]. In ID, circulating levels of T_4 and T_3 remain generally lower and higher respectively than in iodine-sufficient populations. Pituitary gland generally discharges TSH in response to circulating T_4 levels, and circulating TSH rises and falls when circulating T_4 levels remain low and high respectively. Iodine

deficiency reduces T_4 and raises TSH in circulation, so circulating TSH remains generally higher in iodine-deficient populations than in iodine-sufficient ones, and is extensively used as an indicator for both hypothyroidism and hyperthyroidism [11].

Prolonged TSH action forms goiter causing hypertrophy and hyperplasia of thyroid gland. On long standing, initial diffuse goiter becomes nodular and toxic with the appearance of hyperactive autonomous nodules producing excessive T_4 independent of TSH [1]. In addition to goiter and hypothyroidism, ID may also (a) result in mental retardation, deaf-mutism, squint, spastic diplegia, impaired learning capacity, and cretinism, (b) affect reproductive function leading to increased incidence of stillbirths, spontaneous abortions, congenital anomalies, low birth weights, and infant and young child mortality, (c) cause irreversible brain damage in fetus, infant and young children [12], and (d) effectuate short stature, delayed physical development, iodine-induced hyperthyroidism (IIH), and increased susceptibility to nuclear radiation [11]. Goiter increases risk of thyroid cancer, and a large goiter may obstruct trachea and esophagus [1]. All these disorders are known as iodine deficiency disorders (IDD). A total goiter rate (TGR) of ≥ 5% in school-age children of an area reflects the risk of developing IDD in the populations of that area [12]. Major strategies to control ID are implementing universal salt iodization (USI) and monitoring iodine intake periodically [11]. However, more than adequate and excess iodine intakes as evidenced by UI levels of 200–299 µg/L and ≥ 300 µg/L respectively may also cause hypothyroidism [13]. Excess iodine intake may also produce autoimmune thyroid diseases [11].

In the context of thyroid function, Tripura's tribal population has special relevance, because the State of Tripura is in the goiter endemic belt of India [14] and iodine deficient [15], and found goiter endemic [16–19]. Besides, effectiveness of prophylactic USI program introduced in the State prohibiting the sale of non-iodized edible salt on 31st August, 1988 [20] was not satisfactory [19]. Furthermore, Tripura's tribal population often consumes BS [21] rich in antithyroid constituents [22]. But information on the status of thyroid functions in the tribal population was found scanty. Accordingly, objectives of the study were to assess TV, thyroid pathophysiology, TH, TSH, Tg-Ab, TPO-Ab, UI, USCN, SI, WI, BS consumption pattern, and their inter-relationships in Tripura's tribal children.

Materials And Methods

Study Areas

Three hilly villages Deorachhara, Unakoti and Naithangchhara were randomly selected from Tripura Tribal Autonomous District Council. Deorachhara and Unakoti are in Unakoti district, and Naithangchhara is in Dhalai district. All villages are sparsely populated and under USI program, besides all are similar in climate, economy, culture, education, dietary habits and available health facilities.

Study Population

School-aged children are a useful target population for IDD's assessment and surveillance because of their ability to reflect IDD's current status [12]. Therefore, native-born children aged 6–12 years, who were free from diseases, were targeted from tribal populations who traditionally consume BS. In selected villages, 127 children (59 boys and 68 girls) were randomly selected from willing target children. The study was conducted during 2015–2018. Selected children's demographic characteristics have been shown in Table 1.

Thyroid Ultrasonography

Echogenicity of thyroid gland was studied for detecting nodules and measuring TV following WHO's ultrasonographic guidelines [11], using a portable compact Color Doppler Ultrasound Scanner System with a 7.5 MHz linear array transducer (GE LOGIQ E BT 12 Console with 12 L RS Linear Probe, Wipro GE Healthcare Pvt. Ltd) by a radiologist. Each thyroid lobe's volume (in milliliters) was calculated by the formula: 0.479 x (length in centimeters) x (width in centimeters) x (depth in centimeters). Thyroid volume was calculated by adding volumes of right and left lobes. Thyroid volume of each child was compared with 97th percentile reference TV of iodine-sufficient children aged 6–12 years [11] to diagnose the child's thyroid gland as goitrous in case the child's TV exceeded the reference TV for the child's sex and age.

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Serum levels of FT_4, FT_3, TSH, Tg-Ab and TPO-Ab

Serum was separated within 2 hours of fasting venous blood (5 ml) collection from each subject and stored at -20^{o}C until analysis. Serum levels of FT_4, FT_3, TSH, Tg-Ab and TPO-Ab were measured by ELISA microwell methods using kits of FT_4 (Product Code: 1225 – 300, Accu-Bind ELISA Microwells, Monobind Inc., Lake Forest, CA 92630, USA), FT_3 (Calbiotech Inc., 10461 Austin Dr. Spring Valley, CA, 91978), TSH (Product Code: 325 – 300, Accu-Bind ELISA Microwells, Monobind Inc., Lake Forest, CA 92630, USA), anti-Tg-Ab (EUROIMMUN, Medizinische, Labordiagnostika, AG) and anti-TPO-Ab (EUROIMMUN, Medizinische, Labordiagnostika, AG) respectively, following manufacturers' protocols. Analyses of serum levels of FT_4, FT_3, TSH, Tg-Ab and TPO-Ab were completed within 7 days of blood collection.

UI levels

Urine samples (each of about 50 ml) were collected from the selected children in wide-mouthed, screw capped, plastic bottles (adding a drop of toluene to each) and stored at 4^{o}C until analysis. In all, 127 urine samples were collected from three study villages ensuring at least 40 samples from each village, as urine samples collected at random from 40 subjects of an area represent the iodine nutrition status of that locality [23]. Iodine content of collected urine samples were assayed following the method of Karmarkar *et al* [24]. The procedure included: taking 0.1 ml of each urine sample, double distilled water (for a blank) and each working iodine standard in separate test tubes; adding 0.3 ml of 2.5 N potassium carbonate; drying overnight at 80 to 100°C; ashing in muffle furnace at 600^{o}C for 2 hours; adding 3 ml of 0.003 N sodium meta-arsenite on cooling; centrifuging at 2500 g for 7 minutes; incubating supernatant for 5 minutes at 56^{o}C; adding 3 ml of 0.005 N ceric ammonium sulphate at 30 second intervals; mixing; after 20 minutes recording transmittance at 420 nm; and determining urinary iodine by interpolation from standard curve. Estimation of iodine contents of urine samples was completed within 7 days of collection.

USCN levels

The dietary intake of thiocyanate in a population is determined from the daily urinary excretions and urinary concentrations of thiocyanate [25]. Thiocyanate contents of 127 collected urine samples (ensuring at least 40 samples from each village) were estimated following the method of Aldridge [26] as modified by Michajlovskij and Langer [27]. Analyses of thiocyanate contents of urine samples were done within 7 days of collection.

SI levels

Iodized salt samples (each of about 50 g) were collected from the families of the all selected children in the marked screw capped wide mouthed plastic bottles, ensuring at least 35 salt samples from each village, as 35 salt samples collected randomly from an area can reflect the status of USI in that area [28]. Collected salt samples were stored in cool and dark places until analysis. Iodine contents of iodized salt samples were estimated following iodometric titration method [11] within 7 days of collection.

WI levels

Samples (each of about 50 ml) of water used for drinking and house-hold works were collected in wide-mouthed, screw capped, plastic bottles from the families of all children and stored at 4^{o}C until analysis. Water samples were assayed following the method of Karmarkar *et al* [24]. The method involved: taking 7 ml of each water sample, double distilled water (for a blank) and each working iodine standard in separate test tubes; adding 1 ml of 20% sodium chloride, 0.5 ml of 60% sulphuric acid and 0.5 ml of 0.1 N arsenous acid; mixing; keeping in a water bath at 30^{o}C; adding 1 ml of ceric ammonium sulphate; exactly after 20 minutes adding 1 ml of ferrous ammonium sulphate (to stop the reaction) and 0.5 ml of 4% potassium thiocyanate; recording transmittance at 550 nm; and determining WI by interpolation from standard curve. Iodine contents of water samples were analyzed within 7 days of collection.

BS Consumption

To determine per capita daily BS consumption in selected villages, 61 randomly selected ST families consisting of 270 members were interviewed regarding their consumed BS's types and forms, duration of BS availability in a year, consumption frequency per

week, consumption amount per serve, number of family members, and the information thus obtained was recorded.

Ethical Clearance

The study got approval from the Institutional Human Ethical Committee, Department of Physiology, University of Calcutta, Kolkata, India (IHEC/PHY/CU/H-P 34/13, dated 3rd October, 2013). Informed consents of the studied children were obtained in writing from their guardians.

Statistical Analysis

Mean ± SDs, medians and IQRs of age, TV, FT_4, FT_3, TSH, Tg-Ab, TPO-Ab, UI, WI, SI, USCN and BS consumption were calculated using Microsoft Excel 2007. Two tail t-test analysis of 'Two Samples assuming Unequal Variance tool' of Microsoft Excel Analysis ToolPak (MSEATP) was used to calculate mean differences' significance levels (*p*-value). To obtain correlation coefficients (r) as multiple R and their *p*-value, and to prepare regression lines of Fig. 1, MSEATP's 'Regression Analysis Tool' was used.

Results

Mean ± SDs, medians and IQRs of age, TV, FT_4, FT_3, TSH, Tg-Ab, TPO-Ab, UI, USCN, SI and WI are shown by villages and total in Table 1. All children had normal TV and 0.8% children had a thyroidal nodule.

Table 1

Demographic characteristics, and levels of TV, FT_4, FT_3, TSH, Tg-Ab, TPO-Ab, UI, WI, SI, USCN and BS consumption by village and overall, and significance levels of differences between the means of the villages in tribal children of Tripura

Variables	Deorachhara (DC) (*n* = 45) (M/F = 0.80)	Unakoti (UK) (*n* = 41) (M/F = 0.78)	Naithangchhara (NC) (*n* = 41) (M/F = 1.05)	Overall (*n* = 127) (M/F = 0.87)	Significance level (*p-value*) of difference between the means*		
	Mean ± SD/ Median/IQR	Mean ± SD/ Median/IQR	Mean ± SD/ Median/IQR	Mean ± SD/ Median/IQR	DC vs. UK	UK vs. NC	DC vs. NC
Age (y)	9.01 ± 2.01/9.56 /7.18–11	8.37 ± 1.45/8.24 /7.22–9.22	8.68 ± 1.49/8.75 /7.97–9.26	8.69 ± 1.69/8.77 /7.22–9.74	0.091 (NS)	0.344 (NS)	0.404 (NS)
TV (ml)	0.85 ± 0.34/0.86 /0.58–1.02	0.83 ± 0.23/0.85 /0.69–0.94	0.79 ± 0.20/0.76 /0.68–0.85	0.82 ± 0.27/0.82 /0.67–0.96	0.652 (NS)	0.395 (NS)	0.254 (NS)
FT_4 (pmol/L)	22.8 ± 6.1/22.1 /20.8–23.9	19.2 ± 4.8/19.7 /16.9–21.4	16.3 ± 3.5/17.0 /13.3–18.7	19.5 ± 5.6/20.1 /16.9–22.0	0.003 (S)	0.002 (S)	< 0.001 (S)
FT_3 (pmol/L)	4.11 ± 1.21/4.20 /3.27–4.89	5.05 ± 1.32/4.92 /4.30–5.91	3.66 ± 0.70/3.54 /3.14–3.98	4.27 ± 1.24/4.22 /3.35–4.96	0.001 (S)	< 0.001 (S)	0.037 (S)
TSH (mIU/L)	2.66 ± 1.23/2.13 /1.86–3.29	3.25 ± 1.34/3.02 /2.3–3.51	1.40 ± 0.90/1.31 /0.75–1.78	2.44 ± 1.39/2.23 /1.60–3.15	0.037 (S)	< 0.001 (S)	< 0.001 (S)
Tg-Ab (IU/ml)	12.2 ± 2.5/11.5 /10.8–12.5	13.1 ± 5.2/12.0 /11.1–13.0	20.6 ± 15.4/14.7 /12.5–18.6	15.2 ± 10.0/12.5 /11.1–14.5	0.339 (NS)	0.005 (S)	0.001 (S)
TPO-Ab (IU/ml)	4.39 ± 1.59/4.05 /3.44–4.81	4.61 ± 2.04/4.15 /3.38–4.81	7.86 ± 21.40/1.40 /0.1–7.83	5.58 ± 12.25/3.89 /2.90–5.11	0.586 (NS)	0.339 (NS)	0.307 (NS)
UI (µg/L)	117.8 ± 69.3/103 /77–157	130.7 ± 49.9/143 /89–168	103.4 ± 51.0/100 /63–140	117.4 ± 58.5/108 /73–160.5	0.323 (NS)	0.017 (S)	0.273 (NS)
WI (µg/L)	2.18 ± 2.91/1.1 /0.75–2.5	6.32 ± 5.54/4.5 /2–9	5.83 ± 3.79/5 /3–8	4.69 ± 4.56/3 /1.35–7	< 0.001 (S)	0.643 (NS)	< 0.001 (S)
SI (ppm)	22.2 ± 11.0/26.6 /14.4–30.5	20.5 ± 12.3/20 /15.7–25.5	21.0 ± 7.4/21.0 /18.2–23.7	21.2 ± 10.4/20.7 /16.1–28.6	0.513 (NS)	0.841 (NS)	0.549 (NS)
USCN (mg/dl)	1.08 ± 0.67/0.9 /0.6–1.2	0.95 ± 0.36/0.9 /0.7–1.2	0.92 ± 0.88/0.5 /0.3–1.1	0.99 ± 0.67/0.8 /0.5–1.2	0.232 (NS)	0.845 (NS)	0.327 (NS)

Variables	Deorachhara (DC) (*n* = 45) (M/F = 0.80)	Unakoti (UK) (*n* = 41) (M/F = 0.78)	Naithangchhara (NC) (*n* = 41) (M/F = 1.05)	Overall (*n* = 127) (M/F = 0.87)	Significance level (*p-value*) of difference between the means*		
BS (g/person /day)	125.6 ± 78.4/83.3 /71.4–154.8 (FN = 15)	194.4 ± 118.0/166.7 /99.5–250 (FN = 23)	131.7 ± 86.0/119 /65.6–160.7 (FN = 23)	153.8 ± 101.4/119.0 /71.4–214.3 (FN = 23)	0.037 (S)	0.046 (S)	0.823 (NS)

Note: TV, Thyroid volume; FT_4, Free thyroxine; FT_3, Free triiodothyronine; TSH, Thyroid stimulating hormone; Tg-Ab, Thyroglobulin antibody; TPO-Ab, Thyroid peroxidase antibody; UI, Urinary iodine; WI, Water iodine; SI, Salt iodine; USCN, Urinary thiocyanate; BS, Bamboo shoot; IQR, Interquartile range; *n*, Number; M/F, Male/Female ratio; FN, Families' number; S, Significant; NS, Not significant.

* Significance levels of differences between the means of the concerned villages for all variables at α = 0.05 of two-tail t-test.

There were no significant differences between means of boys and girls for the variables (Table 2).

Table 2

Age, and levels of TV, FT_4, FT_3, TSH, Tg-Ab, TPO-Ab, UI, WI, SI and USCN by sex and significance levels (*p*-value) of difference between the means of boys and girls in tribal children of Tripura

Variables	Boys (*n* = 59)	Girls (*n* = 68)	*p*-value of difference between the means of boys and girls*
	Mean ± SD/Median/IQR	Mean ± SD/Median/IQR	
Age (y)	8.76 ± 1.71/8.77/7.23 – 9.85	8.64 ± 1.69/8.76/7.22 – 9.87	0.6814 (NS)
TV (ml)	0.85 ± 0.27/0.83/0.7 – 1.02	0.80 ± 0.26/0.82/0.65 – 1.02	0.3098 (NS)
FT_4 (pmol/L)	20.1 ± 6.8/20.85/17.4 – 22.1	19.0 ± 4.3/19.0/16.2 – 22.0	0.2922 (NS)
FT_3 (pmol/L)	4.28 ± 1.25/4.30/3.38 – 4.05	4.26 ± 1.24/4.11/3.27 – 5.10	0.9308 (NS)
TSH (mIU/L)	2.50 ± 1.49/2.13/1.56 – 3.19	2.39 ± 1.31/2.26/1.71 – 3.19	0.6754 (NS)
Tg-Ab (IU/ml)	14.5 ± 7.6/12.2/11.1 – 14.2	15.9 ± 11.8/12.5/11.2 – 14.3	0.4128 (NS)
TPO-Ab (IU/ml)	5.41 ± 5.43/4.3/3.16 – 6.72	5.73 ± 16.02/3.52/2.82 – 6.75	0.8785 (NS)
UI (µg/L)	115.9 ± 59.1/106/70.5 – 160.5	118.6 ± 58.3/108.5/80.5 – 161.3	0.7962 (NS)
WI (µg/L)	4.53 ± 4.74/2.5/1.35 – 5.75	4.84 ± 4.42/3.25/1.4 – 5.88	0.7080 (NS)
SI (ppm)	20.9 ± 10.9/19.9/15.7 – 28.6	21.6 ± 10.0/21.8/16.2 – 28.7	0.7197 (NS)
USCN (mg/dl)	0.99 ± 0.69/0.8/0.5 – 1.2	0.98 ± 0.65/0.8/0.58 – 1.2	0.9714 (NS)

Note: TV, Thyroid volume; FT_4, Free thyroxine; FT_3, Free triiodothyronine; TSH, Thyroid stimulating hormone; Tg-Ab, Thyroglobulin antibody; TPO-Ab, Thyroid peroxidase antibody; UI, Urinary iodine; WI, Water iodine; SI, Salt iodine; USCN, Urinary thiocyanate; IQR, Interquartile range; *n*, Number; NS, Not significant.

*Significance level of difference between the means of boys and girls at α= 0.05 of two-tail t-test.

Table 3

represents distribution of TSH, FT_4, FT_3, Tg-Ab, TPO-Ab, UI, WI and SI under different ranges. As per manufacturers' reference values, normal TSH, FT_4 and FT_3 levels were observed in 93.7%, 92.9% and 81.1% children, and positive TPO-Ab prevalence was 0.8%. Results have shown that 6.3% children had thyroid dysfunction. Levels of UI of < 100 and ≥ 100 µg/L were observed in 45.7% and 54.3% children respectively. Levels of WI were below > 20 µg/L in all samples, and SI levels found ≥ 15 ppm (parts per million) iodine in 80.3% samples.

Variables	Boys (*n* = 59)	Girls (*n* = 68)	*p*-value of difference between the means of boys and girls*
	Mean ± SD/Median/IQR	Mean ± SD/Median/IQR	
Age (y)	8.76 ± 1.71/8.77/7.23–9.85	8.64 ± 1.69/8.76/7.22–9.87	0.6814 (NS)
TV (ml)	0.85 ± 0.27/0.83/0.7–1.02	0.80 ± 0.26/0.82/0.65–1.02	0.3098 (NS)
FT_4 (pmol/L)	20.1 ± 6.8/20.85/17.4–22.1	19.0 ± 4.3/19.0/16.2–22.0	0.2922 (NS)
FT_3 (pmol/L)	4.28 ± 1.25/4.30/3.38–4.05	4.26 ± 1.24/4.11/3.27–5.10	0.9308 (NS)
TSH (mIU/L)	2.50 ± 1.49/2.13/1.56–3.19	2.39 ± 1.31/2.26/1.71–3.19	0.6754 (NS)
Tg-Ab (IU/ml)	14.5 ± 7.6/12.2/11.1–14.2	15.9 ± 11.8/12.5/11.2–14.3	0.4128 (NS)
TPO-Ab (IU/ml)	5.41 ± 5.43/4.3/3.16–6.72	5.73 ± 16.02/3.52/2.82–6.75	0.8785 (NS)
UI (µg/L)	115.9 ± 59.1/106/70.5–160.5	118.6 ± 58.3/108.5/80.5–161.3	0.7962 (NS)
WI (µg/L)	4.53 ± 4.74/2.5/1.35–5.75	4.84 ± 4.42/3.25/1.4–5.88	0.7080 (NS)
SI (ppm)	20.9 ± 10.9/19.9/15.7–28.6	21.6 ± 10.0/21.8/16.2–28.7	0.7197 (NS)
USCN (mg/dl)	0.99 ± 0.69/0.8/0.5–1.2	0.98 ± 0.65/0.8/0.58–1.2	0.9714 (NS)

Note: TV, Thyroid volume; FT_4, Free thyroxine; FT_3, Free triiodothyronine; TSH, Thyroid stimulating hormone; Tg-Ab, Thyroglobulin antibody; TPO-Ab, Thyroid peroxidase antibody; UI, Urinary iodine; WI, Water iodine; SI, Salt iodine; USCN, Urinary thiocyanate; IQR, Interquartile range; *n*, Number; NS, Not significant.

*Significance level of difference between the means of boys and girls at α = 0.05 of two-tail t-test.

Table 3 Distribution of levels of TSH, FT_4, FT_3, Tg-Ab, TPO-Ab, UI, WI and SI under different ranges in tribal children of Tripura

Table 4

Correlation coefficients (r) with the levels of significance (*p-value*) for different variable pairs related to thyroid functions by sex and overall in tribal children of Tripura*

Variables	Ranges			
TSH (mIU/L)				
<0.39 (Low level)*	0.39 – 6.16 (Normal level)*	>6.16 (High level)*		Total
n = 6 (4.7%)	*n = 119 (93.7%)*	*n = 2 (1.6%)*		*n = 127 (100%)*
FT_4 (pmol/L)				
<10.30 (Low level)*	10.30 – 25.74 (Normal level)*	>25.74 (High level)*		Total
n = 3 (2.4%)	*n = 118 (92.9%)*	*n = 6 (4.7%)*		*n = 127 (100%)*
FT_3 (pmol/L)				
<1.80 (Low level)*	1.80 – 5.41 (Normal level)*	>5.41 (High level)*		Total
n = 2 (1.6%)	*n = 103 (81.1%)*	*n = 22 (17.3%)*		*n = 127 (100%)*
Tg-Ab (IU/ml)*				
≥ 100 (i.e. Positive)*	<100 (i.e. Negative)*			Total
n = 0 (0.0%)	*n = 127 (100%)*			*n = 127 (100%)*
TPO-Ab (IU/ml)				
≥ 50 (Positive)*	<50 (Negative)*			Total
n = 1 (0.8%)	*n = 126 (99.2%)*			*n = 127 (100%)*
UI (µg/L)				
<20 (Severe ID)	20- 49 (Moderate ID)	50 – 99 (Mild ID)	100 – 199 (Adequate)	200 – 299 (>Adequate)	Total
n = 6 (4.7%)	*n = 16 (12.6%)*	*n = 36 (28.4%)*	*n = 63 (49.6%)*	*n = 6 (4.7%)*	*n = 127 (100%)*
WI (µg/L)				
<4 (Severe ID)	4-10 (Moderate ID)	>10 – 20 9 (Relative ID)	>20 (Adequate)	Total
n = 73 (57.5%)	*n = 42 (33.1%)*	*n = 12 (9.4%)*	*n = 0 (0.0%)*	*n = 127 (100%)*
SI (ppm)				
<15 (Below adequate)	≥15 (Adequate)	≥30 (Above adequate)		Total
n = 25 (19.7%)	*n = 74 (58.3%)*	*n = 28 (22.0%)*		*n = 127 (100%)*

Note: TSH, Thyroid stimulating hormone; FT_4, Free thyroxine; FT_3, Free triiodothyronine; Tg-Ab, Thyroglobulin antibody; TPO-Ab , Thyroid peroxidase antibody; UI, Urinary iodine; WI, Water iodine; SI, Salt iodine; ID, Iodine deficiency; *n*, Number.

* Values are Measuring Kits' manufacturer reference values.

Pair of variables	Boys (n = 59)		Girls (n = 68)		Total (n = 127)	
	R	*p-value*	R	*p-value*	R	*p-value*
Age & TV	0.6118	< 0.001 (S)	0.4921	< 0.001 (S)	0.5497	< 0.001 (S)
UI & TSH	0.2609	0.046 (S)	0.2842	0.019 (S)	0.2633	0.003 (S)
TPO-Ab & Tg-Ab	0.6824	< 0.001 (S)	0.3318	0.006 (S)	0.3768	< 0.001 (S)
TV & FT_4	0.3285	0.011 (S)	0.1240	0.314 (NS)	0.2466	0.005 (S)
FT_4 & FT_3	0.2606	0.046 (S)	0.1302	0.290 (NS)	0.2006	0.024 (S)
USCN & FT_4	0.2409	0.066 (NS)	0.2710	0.025 (S)	0.2477	0.005 (S)
TSH & FT_4	0.1185	0.372 (NS)	0.3707	0.002 (S)	0.2135	0.016 (S)
TSH & FT_3	0.1325	0.317 (NS)	0.2223	0.068 (NS)	0.1894	0.033 (S)
WI & UI	0.1365	0.303 (NS)	0.2663	0.028 (S)	0.2039	0.021 (S)
Tg-Ab & FT_4	− 0.1670	0.206 (NS)	− 0.2285	0.061 (NS)	− 0.1869	0.035 (S)
Tg-Ab & TSH	− 0.2127	0.106 (NS)	− 0.2191	0.073 (NS)	− 0.2024	0.023 (S)
USCN & TPO-Ab	0.3069	0.018 (S)	− 0.1500	0.222 (NS)	− 0.0370	0.680 (NS)
UI & FT_4	0.0178	0.894 (NS)	0.2598	0.032 (S)	0.1139	0.202 (NS)

Note: TV, Thyroid volume; UI, Urinary iodine; TSH, Thyroid stimulating hormone; Tg-Ab, Thyroglobulin antibody; TPO-Ab, Thyroid peroxidase antibody; FT_4, Free thyroxine; FT_3, Free triiodothyronine; USCN, Urinary thiocyanate; WI, water iodine; *n*, Number; S, Significant; NS, Not significant.

* Variable pairs related to thyroid functions showing at least one significant correlation coefficient are included here, and correlations of WI and SI were tested with UI only.

Regression lines for prediction of UI, TSH, FT_4, FT_3, TPO-Ab, TV from the related variables are shown in Fig. 1.

Dietary survey on BS has shown that overall mean ± SD, median and IQR value of BS consumption were 153.8 ± 101.4, 119.0 and 71.4–214.3 g/person/day respectively (Table 1) for about 6 months from May to October. Studied families mainly consumed muli (*Melocanna baccifera*), mritinga (*Bambusa tulda*), rupai (*Dendrocalamus longispathus*), dalu (*Schizostachyum dullooa*), barak (*Bambusa balcooa*) and jai or bari (*Bambusa vulgaris* var.*vulgaris*) types of BS in cooked forms as curry, fry, prickle and boiled BS. Curry mainly included muya awandru (main ingredients are bamboo shoot, rice flour and fermented dry fish), muya bai wahan (main ingredients are bamboo shoot, jackfruit, papaya and pork), muya chakhui (main ingredients are bamboo shoot, papaya, pulses and baking soda) and muya gudak (main ingredients are bamboo shoot and fermented dry fish)

Unakoti village having highest levels of BS consumption pattern (p = 0.037) and median UI showed higher levels of TSH (p = 0.037) and FT_3 (p = 0.001) compared to Deorachhara of the lowest BS consumption (Table 1).

Discussion

The study reveals the status of thyroid functions in BS consuming Tripura's tribal children assessing their TV, thyroid pathophysiology, TH, TSH, Tg-Ab, TPO-Ab, UI, USCN, SI, WI and BS consumption.

WI and its relation with UI

Bioavailability of iodine in an area is generally reflected in its WI. An area is considered as a severe, moderate or relative iodine deficient zone or iodine sufficient zone depending on whether WI content is < 4, 4–10, > 10–20 or > 20 µg/L respectively [29]. As per the criteria, studied villages were in severe to moderate iodine deficient zones, in overall being severe iodine deficient (Table 1)

and found consistent with our previous report [15]. However, there was a positive correlation between WI and UI (r = 0.2039, p = 0.021) in the studied children (Table 4) as observed also by Lv *et al* [30]. All these suggest that though the studied villages were iodine deficient, foodstuffs available in the villages were significant sources of the consumed iodine.

SI and its relation with UI

Earlier studies have reported both positive [19] and no [30] associations between SI and UI; however, the present observations are found consistent with the views of the latter. Besides, the ongoing USI program has yet to achieve the recommended adequacy level of ≥ 15 ppm iodine in ≥ 90% salt samples [11]. However, the USI program under present study was better in effectiveness than that of 1996-98 showing iodine adequacy level in about 63% salt samples [19] and, all studied villages had WHO/UNICEF/ICCIDD recommended median UI levels of 100–199 µg/L [11] indicating adequate iodine nutrition in the population of studied villages. In all, 54.3% of children studied had UI levels of ≥ 100 µg/L, and 45.7% had UI levels of < 100 µg/L (Table 3) which may be explained by the differences in hydration rather than by true ID [31]. All these indicate that the introduced prophylactic USI program was not effective enough in the villages and consequently was not a significant source of consumed iodine for studied children.

UI and its relations with TSH, FT_4 and FT_3

Previous studies on the association of iodine intake level with the levels of T_4, T_3 and TSH conflict with each other and thereby suggest no clear relation of iodine intake level with the levels of T_4, T_3 and TSH [13, 32–35].

In present study, UI was positively related with TSH (r = 0.2633, p = 0.003) which, in turn, remained positively associated with FT_4 (r = 0.2135, p = 0.016) and FT_3 (r = 0.1894, p = 0.033) (Table 4) indicating TSH-dependent increase in TH by consumed iodine in studied children. A positive relation of UI with FT_4 (r = 0.2598, p = 0.032) in girls (Table 4) indicates that consumed iodine also increased FT_4 independent of TSH in girls. The finding as mentioned was found consistent with the views of an *in vitro* study that iodine increases TH, stimulating tyrosine iodination and coupling reactions, by binding to a limited number of high-affinity sites on TPO [36].

TSH and its relations with FT_4 and FT_3

Means of FT_4 and FT_3 as noted (Table 1) were higher and lower respectively than that of a study with excessive iodine intake [32], and higher in both cases than that of a study having adequate iodine intake [33]. Median TSH as observed (Table 1) was lower than that of the studies with excessive iodine [32], and adequate iodine [33]. Low FT_4 levels (hypothyroxinemia) and high FT_4 levels (hyperthyroxinemia) were observed in 2.4% and 4.7% children respectively (Table 3), and remained associated with normal TSH levels. Low FT_3 levels (hypotriiodothyroninemia) and high FT_3 levels (hypertriiodothyroninemia) were observed in 1.6% and 17.3% children respectively (Table 3), and remained associated with normal TSH levels. The possible cause of higher hypertriiodothyroninemia prevalence as observed may be for TSH's positive association with FT_3. It has been also reported that high-carbohydrate diets remain positively associated with higher triiodothyronine levels, and higher triiodothyronine levels were related to increased iodine requirement. Increased iodine requirement exceeding iodine availability may cause IDD [37]. Low TSH levels (hypothyrotropinemia) and high TSH levels (hyperthyrotropinemia) were found in 4.7% and 1.6% children respectively (Table 3), and remained associated with normal FT_4 and FT_3 levels. Overall prevalence of hyperthyrotropinemia in present study (Table 3) was lower compared to that of iodine excess areas [32].

Available literature classifies hypothyroidism into subclinical (high TSH and normal FT_4), primary or overt (high TSH and low FT_4) and secondary or central (low TSH and low FT_4), and hyperthyroidism into subclinical (low TSH and normal FT_4), primary (low TSH and high FT_4) and secondary or TSH-mediated (high TSH and high FT_4) [38, 39]. Present study's thyroid dysfunction prevalence (1.6% subclinical hypothyroidism and 4.7% subclinical hyperthyroidism) was lower than that of the area with adequate iodine [33]. Positive associations of TSH with FT_4 and FT_3 as observed (Table 4) suggest that levels of FT_4 and FT_3 were below their set points which led to compensatory increase in TSH that subsequently raised the levels of FT_4 and FT_3 in

studied children. Further, positive association between FT_4 and FT_3 (r = 0.2006, p = 0.024) as observed (Table 4) suggests that FT_4 has increased FT_3 in studied children.

Thyroid gland and its relation with age and FT_4

Thyroid gland is considered goitrous when its volume exceeds 97th percentile thyroid volume of iodine-replete population, and TGR of 0.0-4.9, 5.0-19.9, 20.0-29.9 and ≥ 30% indicate 'no', 'mild', 'moderate' and 'severe' IDD respectively [11]. Besides, prevalence of thyroid nodules in children may generally vary within 1.0-1.5% [40]. Present study showing all children with normal TV and 0.8% children with a thyroidal nodule suggests that IDD is not a major problem in studied villages. However, the present study's TV (Table 1) was lower than that of the excess iodine-exposed area where IDD based on TGR was a moderate public health problem [32]. A cohort study reported that TV was correlated positively with FT_4 in both sexes' euthyroid adults, and negatively with age in females [41]. However, positive associations of present study's TV with age (r = 0.5497, p = < 0.001) and FT_4 (r = 0.2466, p = 0.005) (Table 4) suggest that TV increased with age, and increase in TV elevated FT_4 in studied children.

Thyroid autoantibodies and their relations with UI, USCN, TSH and FT_4

Literature showing both excessive UI and low UI as risk factors [33] and no risk factors [42] for thyroid autoimmunity suggests no clear association of excessive UI and low UI with thyroid autoimmunity. The prevalence of thyroid autoimmunity in children varies in between 0.5-3% in areas having no IDD [42]. However, more than adequate and excessive iodine intake may convert the state of higher Tg-Ab or TPO-Ab to overt hypothyroidism [13]. Median Tg-Ab as found (Table 1) was lower than that of adequate iodine nutrition zone [33], and the prevalence of positive Tg-Ab and positive TPO-Ab (Table 3) were lower than that of excess iodine area [32]. Negative associations of Tg-Ab as observed with TSH (r=-0.2024, p = 0.023) and FT_4 (r=-0.1869, p = 0.035) (Table 4) suggest that Tg-Ab increased the risk of central hypothyroidism while positive association of Tg-Ab with TPO-Ab (r = 0.3768, p = < 0.001) (Table) as found further indicates that Tg-Ab and TPO-Ab levels were related in such a way that they increased each other in studied children.

Besides, thiocyanate in excess may cause or aggravate endemic goiter in both iodine deficient [43] and iodine sufficient regions [44]. It has been reported further that in ID, higher serum thiocyanate (≥1 mg/dl: at which thiocyanate inhibits iodine pump) associated with USCN of ≥1.9 mg/dl remain associated with lower T_4 and higher TSH, and thyroid hyperplasia [43]. However, the present study showing USCN's positive relation with FT_4 (r = 0.2477, p = 0.005) (Table 4) indicates that thiocyanate has increased FT_4 in studied children. This supports the observation of an *in vitro* study that thiocyanate at low concentration (upto 1 mmol/L) can mimic iodine's stimulatory effect on coupling reactions associated with TH synthesis [36]. Present study showing positive correlation between USCN and TPO-Ab (r = 0.3069, p = 0.018) in boys (Table 4) suggests that thiocyanate increases the risk of central hypothyroidism in studied boys, by increasing Tg-Ab through TPO-Ab. This observation further confirms that thiocyanate results in autoimmune thyroid diseases [45].

BS consumption and thyroid functions

Daily BS consumption in Tripura's ST population during BS-growing seasons found about 120–150 g/person (Table 1). Accordingly, BS consumption was much more than that of Australia and New Zealand (range 31–70 g/day/person) [46]. Moreover, BS has been reported to contain antithyroid cyanogenic glycosides like taxiphyllin [46], thiocyanate, glucosinulate [22], 2,4-dihydroxy benzoic acid [47], etc. Thiocyanate decreases thyroidal iodine uptake [46] and 2,4-dihydroxy benzoic acid inhibits TPO activity [48]. Bamboo-shoots are found to increase TV and UI excretion, to reduce TPO activity, T_4 and T_3 levels in rats [49], and to decrease major enzyme activities of thyroid hormone synthesis in cultured thyrocytes [50]. Present study showing (i) children's maximal FT_4 in Deorachhara where consumption of BS was lowest and intake of iodine was adequate (Table 1), and (ii) children's maximal levels of TSH and FT_3 in Unakoti where BS consumption was highest and iodine intake was adequate (Table 1) – suggests that BS might have interfered with thyroid function in studied children. However, it has already been reported that the antithyroidal action of BS can be mitigated partially by iodine [49, 51].

On the whole, it may be concluded that Tg-Ab tends to reduce the levels of both FT_4 and TSH in the children having adequate iodine nutrition, and in this condition (i) FT_4 and FT_3 remain up-regulated by TSH upsurge which is associated with rise in iodine

intake, (ii) Tg-Ab is enhanced by TPO-Ab and may be increased by thiocyanate through TPO-Ab and (iii) consumed BS probably induces iodine-dependent elevation of TSH, and possibly contributes to the levels of FT_3 and thiocyanate in studied children. Thus, it appears that studied children are at risk of developing hypothyroidism that may partially be reduced by decreasing BS consumption.

Declarations

Author contributions

I.R. – Funding and resources acquisition as Tripura project administrator, methodology, investigation, data acquisition, statistical analysis and interpretation of data, and manuscript preparation; A.K.C. – Original conceptualization, visualization, supervision, data evaluation and interpretation; S.K.D. – Data evaluation and interpretation ; S.K.M. – Investigation, data evaluation and interpretation; A.D. – Investigation and data acquisition ; L.H.S. – Member project team; and all authors: Writing and editing the final manuscript.

Conflict of Interest

No conflict of interest regarding this article was reported.

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Figures

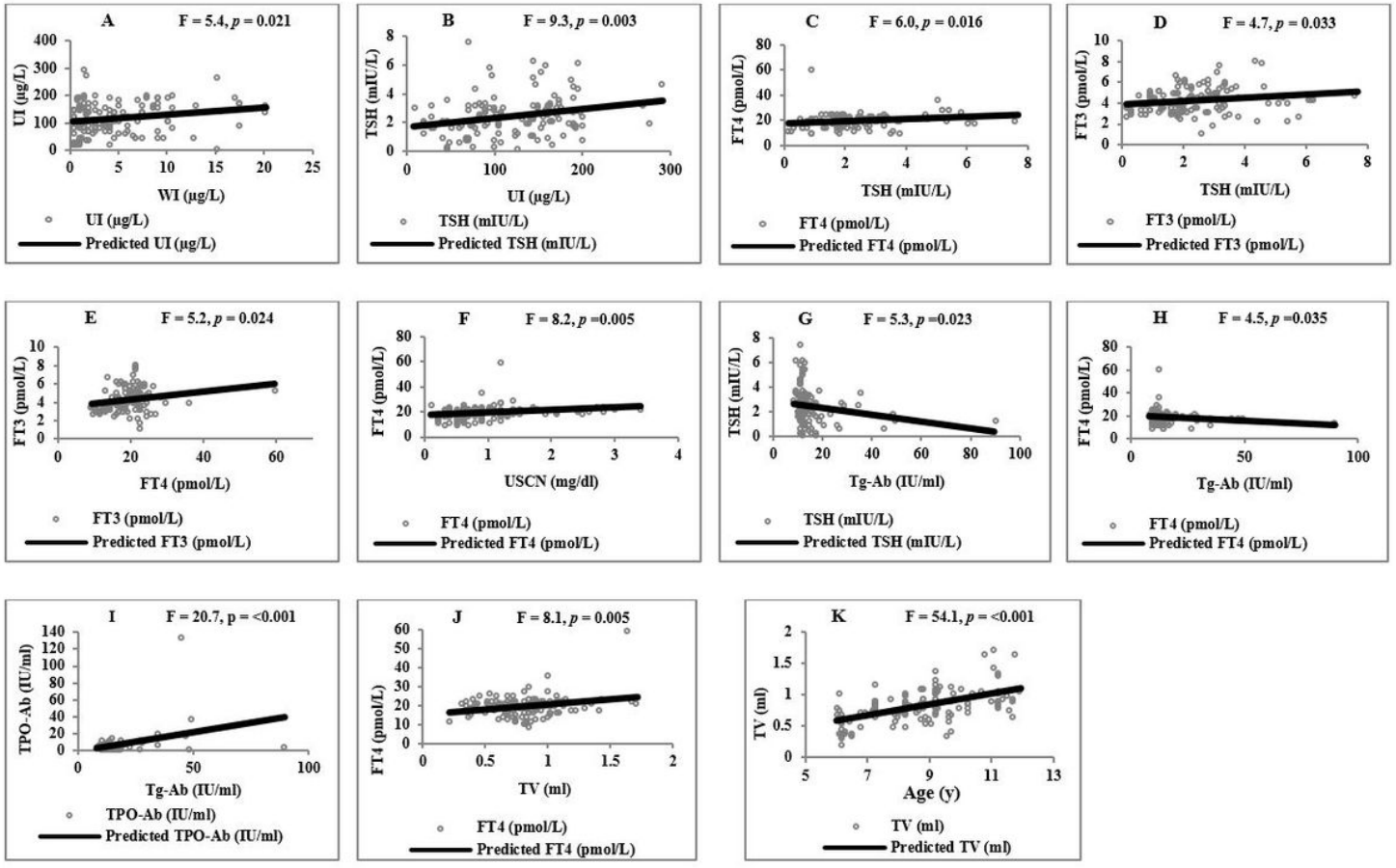

Figure 1

The regression lines of line fit plots for predictions of UI from WI (Panel A), TSH from UI (Panel B), FT4 from TSH (Panel C), FT3 from TSH (Panel D), FT3 from FT4 (Panel E), FT4 from USCN (Panel F), TSH from Tg-Ab (Panel G), FT4 from Tg-Ab (Panel H), TPO-Ab from Tg-Ab (Panel I), FT4 from TV (Panel J), and TV from age (Panel K) in tribal children of Tripura. Note: WI, Water iodine; UI, Urinary iodine; TSH, Thyroid stimulating hormone; FT4, Free thyroxine; FT3, Free triiodothyronine; USCN, Urinary thiocyanate; Tg-Ab, Thyroglobulin antibody; TPO-Ab, Thyroid peroxidase antibody; TV, Thyroid volume.

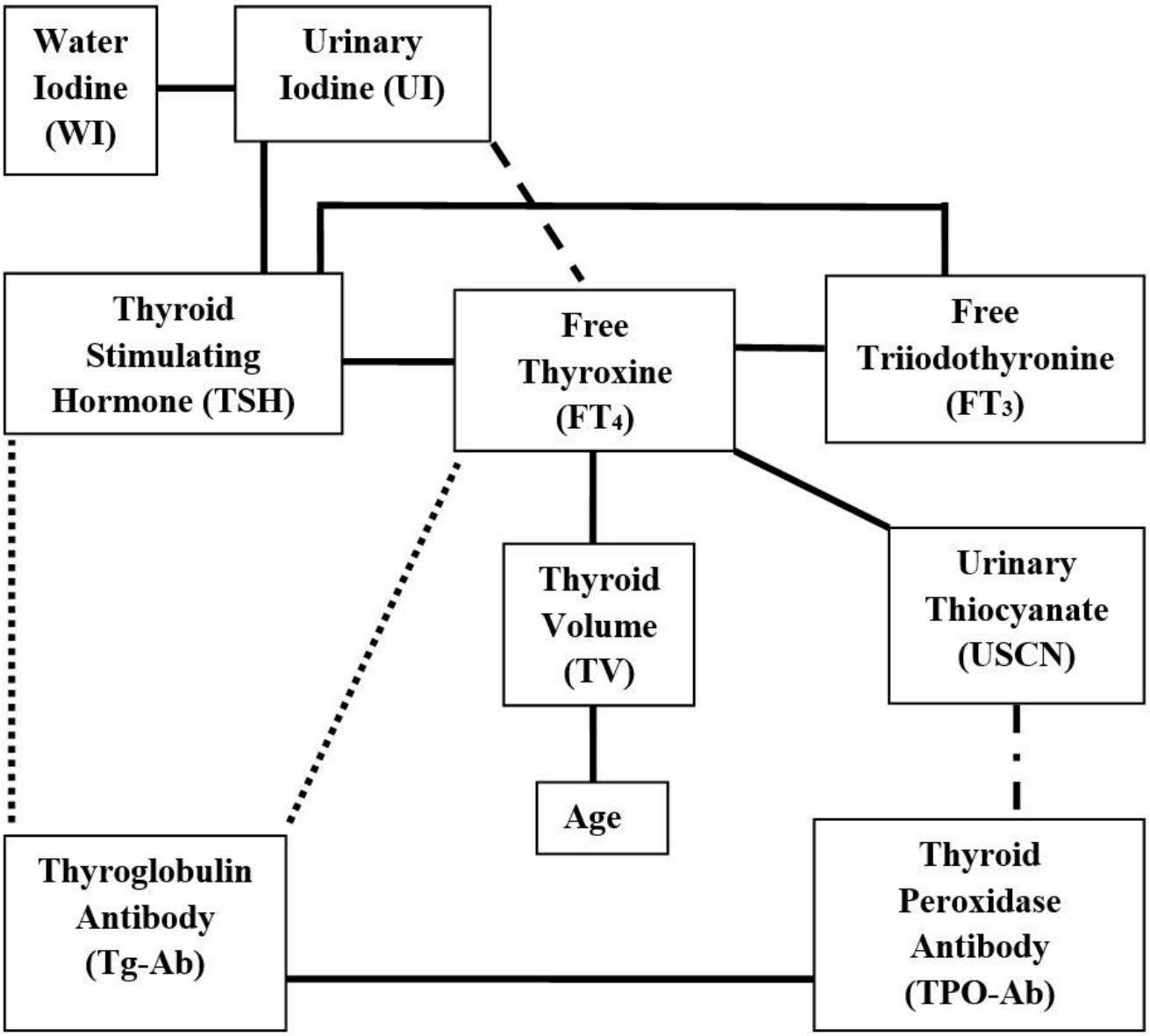

Figure 2

presents a scheme of interrelationships of thyroid related variables. Note: Solid, dash-dot and dash lines represent positive relationships in all children, boys and girls respectively, and dot lines indicate negative relationships in all children.