



Autoimmune thyroid diseases after 25 years of universal salt iodisation: an epidemiological study of Chinese adults in areas with different water iodine levels

Siyuan Wan^{1,2}, Mengying Qu¹, Huaiyong Wu¹, Bingxuan Ren¹, Wen Jiang³, Xiaoming Wang³, Lixiang Liu^{1*} and Hongmei Shen^{1*}

¹Centre for Endemic Disease Control, Chinese Centre for Disease Control and Prevention, Harbin Medical University, Harbin 150081, People's Republic of China

²Department of Preventive Medicine, Qiqihar Medical University, Qiqihar 161006, People's Republic of China

³Shandong Institute of Prevention and Control for Endemic Disease, Jinan 250014, People's Republic of China

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Abstract

The present study aimed to evaluate the status of iodine nutrition and thyroid function in adults, to understand the distribution of thyroid disease in people with autoimmune thyroid disease (AITD) in different water iodine areas and to explore the relationship between serum iodine, urine iodine and thyroid function in people with AITD. A cross-sectional survey was conducted in areas of Shandong Province with different water iodine levels, and subsequently 1225 adults were enrolled from iodine-deficient (ID), iodine-adequate (IA) and iodine-excess (IE) areas. Urinary iodine, water iodine, salt iodine, serum iodine and thyroid function were measured. According to the urine iodine concentration, the ID and IA areas were defined as iodine sufficient and the IE area as iodine excessive. Urine iodine, serum iodine, free thyroxine (FT₄) and thyroid-stimulating hormone (TSH) levels were comparatively higher in the IE area. The positive rate of thyroglobulin antibody (19.1 %) and the prevalence of AITD (21.8 %) were higher in the ID areas; the prevalence of subclinical hypothyroidism was lowest in the ID areas (7.3 %) and highest in the IE area (16.3 %). Among the AITD population, urinary iodine concentration, free triiodothyronine, FT₄ and TSH had a non-linear correlation with serum iodine; abnormal TSH level, serum iodine concentration > 110 µg/l and goitre were risk factors for AITD in adults, especially females. Our data collectively suggest that universal salt iodisation has improved the iodine nutritional status of the population in ID areas in China. Non-step-by-step iodine fortification may induce the transformation of thyroid autoimmune diseases from recessive-to-dominant in susceptible people. Moreover, enhanced monitoring of thyroid function in people with AITD is important.

Key words: Iodine fortification: Urinary iodine: Adults: Serum iodine: Thyroid autoimmunity

Iodine is an essential micronutrient for the human body and an integral component of thyroid hormones. Iodine plays a physiological role in thyroid hormones, promotes the metabolism of substances and energy and promotes growth and development^(1,2). Iodine intake must be within a certain range; if too low or too high, iodine will have adverse effects on the body^(3,4).

Over the past two decades, the universal salt iodisation (USI) strategy has been recognised as a safe, economic and sustainable policy to eliminate iodine deficiency diseases (IDD). China is one of the most seriously affected areas of IDD in the world. In 1995, China began to implement USI, which has played a major role in eliminating IDD^(5,6).

In recent years, there have been a number of reports of iodine overdoses leading to autoimmune thyroiditis^(7–9). At the same time, it has been shown in domestic epidemiological investigations that the nationwide fortification of iodine can also increase the prevalence of thyroid diseases, especially hyperthyroidism^(10–12). Some studies have reported that long-term fortification of iodine after iodine deficiency will have an impact on thyroid autoimmune status^(13,14); however, similar studies are generally small in scale and quantity, and there is a lack of large sample epidemiological studies in China. So, what is the status of thyroid disease in China's iodine-deficient (ID) areas after 25 years of USI? What is the thyroid immune status in this region?

Abbreviations: AITD, autoimmune thyroid disease; FT₃, free triiodothyronine; FT₄, free thyroxine; IA, iodine adequate; ID, iodine deficient; IDD, iodine deficiency disease; IE, iodine excess; MWI, median water iodine; SIC, serum iodine concentration; Tg, thyroglobulin; TGAb, thyroglobulin antibody; TPOAb, thyroid peroxidase antibody; TSH, thyroid-stimulating hormone; UIC, urinary iodine concentration; USI, universal salt iodisation.

* **Corresponding authors:** Hongmei Shen, fax +86 451 86657674, email shenhm119@hrbmu.edu.cn; Lixiang Liu, fax +86 451 86675814, email liuli216@163.com



In the current study, we conducted an epidemiological investigation of abnormal thyroid antibodies in three regions of China with different water iodine contents. The iodine nutritional status of people in the different water iodine areas was determined, and the relationship between serum iodine, urine iodine and thyroid function in patients with abnormal thyroid antibody was ascertained to provide theoretical bases for the prevention of autoimmune thyroid disease (AITD).

Materials and methods

Survey areas and subjects

In this survey, three geographic areas with different iodine levels were selected, including ID areas with a median water iodine (MWI) level $<10 \mu\text{g/l}$, iodine-adequate (IA) areas with a MWI between 40 and $100 \mu\text{g/l}$ and the iodine-excess (IE) areas with a MWI $>300 \mu\text{g/l}$ ⁽¹⁵⁾. A MWI $\leq 10 \mu\text{g/l}$ is defined as an ID area according to the Chinese national standard⁽¹⁶⁾. Iodised salt was provided in the ID areas included in this survey, and the coverage of household use of qualified iodised salt was $>90\%$ according to the Chinese national standard⁽¹⁷⁾. Iodised salt was not provided in the IA and IE areas.

A multi-stage stratified random sampling method was used in this study. Based on the available data from the most recent national IDD surveillance and water iodine surveillance in China⁽¹⁸⁾, Shandong Province was selected as the survey area. In the first stage, one district/county was randomly selected from the three different water iodine regions in Shandong Province. In the second stage, one town was randomly selected in each district/county. In the third stage, according to the expected sample size, 1–2 villages were randomly selected from each town. In the final stage, individuals meeting the inclusion criteria in the selected villages were randomly selected as the subjects of the study.

Based on the sampling method, Dongtan and Qianlv villages in Jiaxiang County located in Jining City were selected as the ID areas. Liuxiangzhuang and Dongding villages in Weishan County of Jining City were selected as IA areas, and Jieyuanji village in Mudan District of Heze City was selected as the IE area. These villages are located in the southwestern part of Shandong Province in China (Fig. 1).

More than 20 years has elapsed since the USI was implemented in China; the prevalence of AITD varies widely across areas with different water iodine levels^(19–21). At the same time, the prevalence of AITD in the survey areas has not been previously reported. This study was cross-sectional in design, and the sample size was computed using the following formula: $N = Z^2_{\alpha/2} P(1 - P)/d^2$ ($\alpha = 0.05$, $P = 0.5$, $d = 0.05$)⁽²²⁾. Based on the calculation, the expected sample for each area was at least 384 adults.

Finally, a total of 1225 adults were recruited in this study. Of the 1225 adults enrolled in this study, 409, 392 and 424 were from the ID, IA and IE areas, respectively. The recruitment criteria included residing in the survey areas for more than 5 years and 18–60 years of age. To control for confounding factors, the following were excluded: patients with congenital thyroid disease and taking anti-thyroid drugs or thyroxine in the past

year; patients with autoimmune, endocrine, heart, chronic and family genetic diseases; and pregnant women. The three areas mentioned above were villages with similar living standards, dietary habits and environmental conditions.

Survey methods and indicators

A standard questionnaire was used to collect information on the demographic characteristics, including sex, name, age, BMI (weight/height² (kg/m^2)), education level, family income, MWI, current smoking status, alcohol consumption, radiation history (including whether or not radiation examinations have been performed in the past 10 years, whether or not there is a source of radiation near the residence, and the frequency of using computers and mobile phones) and a personal or family history of thyroid disease (including type of thyroid disease). The questionnaire was administered by trained research assistants in face-to-face interviews. The BMI was classified according to the Chinese Public Health Standards as follows: underweight, BMI $<18.5 \text{ kg/m}^2$; normal weight, $18.5 \leq \text{BMI} < 24.0 \text{ kg/m}^2$; overweight, $24 \leq \text{BMI} < 28.0 \text{ kg/m}^2$; and obese, BMI $\geq 28.0 \text{ kg/m}^2$ ⁽²³⁾. According to the WHO, an individual is a smoker if he/she smokes at least one cigarette per day for >1 month, and if a person drinks at least once a week for >1 year, the individual is considered an alcohol consumer.

Based on the recommendation from the WHO, urinary iodine concentration (UIC) and thyroid-stimulating hormone (TSH) levels were used to evaluate the iodine nutrition status of the population⁽²⁴⁾. In view of the limitations of these indicators, free triiodothyronine (FT₃), free thyroxine (FT₄), thyroglobulin antibody (TGAb) and thyroid peroxidase antibody (TPOAb) levels were also measured. In addition, given the sensitivity of serum iodine in evaluating individual iodine nutrition status, the stability of population iodine nutrition status and the significance of screening in people at high risk for thyroid disease, serum iodine was also included for testing⁽²⁵⁾. Thyroglobulin (Tg) is a biomarker of iodine deficiency and iodine nutrition status^(26,27). However, a wide range of methods are used to analyse Tg, making it difficult to compare studies⁽²⁶⁾. Therefore, Tg was not determined in the present study. Moreover, we collected salt samples from all participants for the determination of salt iodine content.

This project was approved by the Ethical Review Board of Harbin Medical University (no. hrbmuecdc20200320). Written informed consent was obtained from all participants before the survey was conducted.

Water sample collection and iodine determination

Before the survey was conducted, water iodine levels were determined to verify whether or not the three areas met the criteria for inclusion in the study. The samples of water were collected according to the Chinese national standard⁽²⁸⁾. Samples of the daily drinking water of participants were collected in clean plastic tubes. For ID areas (Dongtan and Qianlv villages) with a dispersed water supply, five samples each were collected from the east, west, south, north and central locations in each village. In total, ten samples were collected from the ID areas. For IA (Liuxiangzhuang and Dongding villages) and IE (Jieyuanji village) areas with a centralised water supply, two parallel samples

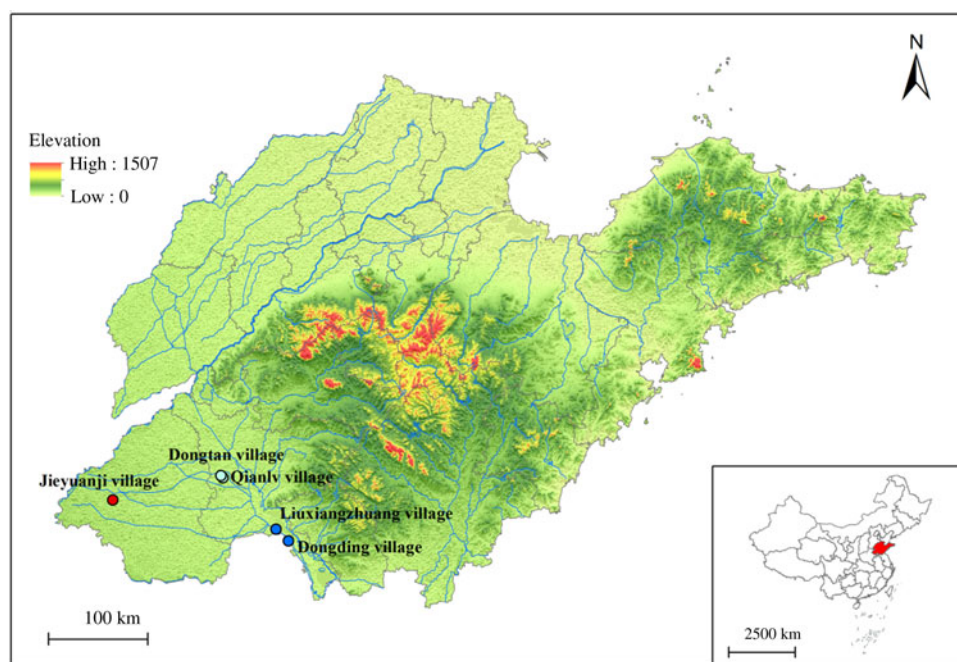


Fig. 1. Geographical distribution of survey areas in Shandong Province of China. ○, Median water iodine (MWI) $\leq 10 \mu\text{g/l}$ (iodised salt fortification); ●, $40 < \text{MWI} < 100 \mu\text{g/l}$; ●, MWI $> 300 \mu\text{g/l}$; —, river; □, city boundary.

(the average value was calculated) were collected from each village, giving a total of six samples from the IA and IE areas. Each water sample was at least 15 ml. The water samples were kept at room temperature, and the determination was completed within 24 h. The iodine concentration of drinking water was determined using the method of As^{3+} – Ce^{4+} catalytic spectrophotometry recommended by the Chinese National Reference Laboratory for Iodine Deficiency Disorders, Chinese Centre for Disease Control and Prevention⁽²⁹⁾.

Salt sample collection and iodine determination

Salt samples were collected from all of the participants in the three areas. Each participant provided at least 50 g of table salt in a clean, labelled ziploc bag. The iodine content in the salt samples was determined using the general test method of the salt industry⁽³⁰⁾. The standard salt iodine content in the ID areas of Shandong Province was 25 mg/kg, and non-iodised salt was supplied in IA and IE areas. The salt type was classified into the following categories: non-iodised salt (salt iodine content $< 5 \text{ mg/kg}$), qualified iodised salt ($18 \text{ mg/kg} \leq \text{salt iodine content} \leq 33 \text{ mg/kg}$) and unqualified iodised salt ($5 \text{ mg/kg} < \text{salt iodine content} < 18$ or $> 33 \text{ mg/kg}$).

Urine sample collection and iodine determination

From each participant, the fasting single-spot urine sample was collected in the morning (08.00–11.00 hours) in clean, well-labelled plastic tubes and stored at 4°C . The test was completed within 2 weeks. UIC was measured according to the China Health Standard Method of Determination of Iodine in Urine by As^{3+} – Ce^{4+} catalytic spectrophotometry⁽³¹⁾.

Blood sample collection and determination

Five millilitres of venous blood (no anticoagulant) was collected from the study subjects and centrifuged at 3000 *g* after standing at room temperature for 2 h. Serum was separated and stored in a low-temperature refrigerator at -80°C .

Serum iodine concentration (SIC) was measured using an inductively coupled plasma-MS system (PerkinElmer NexION 350). The levels of FT_3 , FT_4 , TSH, TPOAb and TGAb were determined using chemiluminescent immunoassay (Siemens Healthcare Diagnostics Inc.).

The reference values were 52–109 $\mu\text{g/l}$ SIC (Quest Diagnostics⁽³²⁾), 3.1–6.8 pmol/l FT_3 , 11.5–22.7 pmol/l FT_4 , 0.27–4.2 $\mu\text{IU/ml}$ TSH, 0–60 U/ml TPOAb and 0–60 U/ml TGAb. In addition, experienced radiologists used portable ultrasound instruments equipped with 7.5 MHz transducers to evaluate thyroid glands. TPOAb and TGAb are of great significance in the diagnosis of autoimmune thyroiditis. Weetman⁽³³⁾ considered that pure TGAb, TPOAb-positive and double antibody-positive indicated a diagnosis of AITD. Thus, the diagnostic criteria for thyroid disease were as follows: hypothyroxinaemia: $\text{FT}_4 < 11.5 \text{ pmol/l}$ and TSH within the normal range; overt hypothyroidism: TSH $> 4.20 \mu\text{IU/ml}$ and $\text{FT}_4 < 11.5 \text{ pmol/l}$; subclinical hypothyroidism: TSH $> 4.20 \mu\text{IU/ml}$ and FT_4 within the normal range; overt hyperthyroidism: TSH $< 0.27 \mu\text{IU/ml}$, $\text{FT}_4 > 22.7 \text{ pmol/l}$ and $\text{FT}_3 > 6.8 \text{ pmol/l}$; subclinical hyperthyroidism: TSH $< 0.27 \mu\text{IU/ml}$, and FT_3 and FT_4 within the normal range; AITD: TPOAb (+)/TGAb (+) (TPOAb- and/or TGAb-positive) and both test values $> 60 \text{ U/ml}$ were positive; and goitre: thyroid volume $> 25 \text{ ml}$ (male) and $> 18 \text{ ml}$ (female). All of the above thyroid diseases, except AITD, include individuals with positive thyroid antibody titers.



Statistical analysis

Statistical analyses were performed using Excel 2016 and SPSS software (version 17.0) for Windows. Non-normally distributed data are expressed as medians and interquartile ranges. The three groups were compared using one-way ANOVA on normally distributed variables. The variables with a skewed distribution were assessed with the Kruskal–Wallis test. If a difference was found between multiple groups, a *post hoc* examination was performed accordingly. The χ^2 test was adopted for comparing the rates between groups with categorical outcomes. A binary logistic regression model was applied to calculate the OR and 95% CI for AITD from the three areas. The model for AITD was adjusted for age, sex and BMI. A $P < 0.05$ was considered statistically significant.

Results

Demographic characteristics of the adults included in this study

The demographic characteristics of the adults in the three areas are presented in Table 1. In this study, the MWI concentration in the ID, IA and IE areas was 2.6, 71.4 and 325.0 $\mu\text{g/l}$, respectively. The coverage of household use of qualified iodised salt in the ID areas was 93.9%, which is in agreement with the evaluation content and criteria for the elimination of IDD in China (coverage of household use of qualified iodised salt $> 90.0\%$)⁽¹⁶⁾. Except for five iodised salt samples detected in IA areas (participants who provided iodised salt will be excluded in later data analyses), other salt samples in the IA and IE areas were in agreement with Chinese national standards (non-iodised salt should be provided to IA and IE areas).

Iodine nutrition status and thyroid function in adults

The UIC and thyroid function status of adults in the three areas are shown in Table 1. The UIC of the adults in the ID, IA and IE areas was 228.4, 243.9 and 390.4 $\mu\text{g/l}$, respectively. According to the recommendations of the WHO/UNICEF/ICCIDD⁽²⁴⁾, the iodine status of ID and IA areas was sufficient, while the iodine status in the IE area was excessive.

The median urinary iodine and SIC, FT_3 , FT_4 , TSH levels, and TGAb-positive rate of adults in the ID, IA and IE areas differed significantly ($H = 160.425$, $P < 0.001$; $H = 32.354$, $P < 0.001$; $H = 13.630$, $P = 0.001$; $H = 82.974$, $P < 0.001$; $H = 17.768$, $P < 0.001$ and $\chi^2 = 15.551$, $P < 0.001$, respectively).

The urine iodine and serum iodine levels in the IE area were significantly higher than the IA areas ($Z = -9.606$, $P < 0.001$ and $Z = -5.664$, $P < 0.001$, respectively), and the serum iodine level in ID areas was significantly higher than the IA areas ($Z = -2.403$, $P = 0.016$). Compared with ID areas, the IE area had higher urinary iodine and serum iodine levels ($Z = -11.920$, $P < 0.001$ and $Z = -3.194$, $P = 0.001$, respectively).

Compared with the ID areas, the FT_3 level in the IE area was significantly lower ($Z = 13.271$, $P < 0.001$). Compared with the levels in the IA and ID areas, the FT_4 level in the IE area was significantly higher ($Z = -6.751$, $P < 0.001$ and $Z = 74.320$, $P < 0.001$, respectively). Compared with the IA areas, the TSH

level in the ID areas was significantly lower ($Z = -3.399$, $P = 0.001$), while the TSH level in the IE area was significantly higher than the ID areas ($Z = 14.809$, $P < 0.001$). No statistical difference was detected in the TPOAb-positive rate and double antibody-positive rate. Compared with the IA areas, the TGAb-positive rate in the ID areas was significantly higher ($\chi^2 = 11.735$, $P = 0.001$), while the TGAb-positive rate in the IE area was significantly lower than the ID areas ($\chi^2 = 9.740$, $P = 0.002$).

Thyroid disease in the three areas

In the ID, IA and IE areas, the prevalence of subclinical hypothyroidism and AITD differed significantly among adults ($\chi^2 = 15.847$, $P < 0.001$ and $\chi^2 = 14.885$, $P = 0.001$, respectively). Compared with the IA areas, the prevalence of subclinical hypothyroidism in the ID areas was significantly lower ($\chi^2 = 4.991$, $P = 0.025$), but significantly higher in the IE area than the ID areas ($\chi^2 = 15.853$, $P < 0.001$). The prevalence of AITD was significantly higher in the ID areas than the IA areas ($\chi^2 = 12.783$, $P < 0.001$). Compared with the ID areas, the prevalence of AITD in the IE area was lower ($\chi^2 = 7.666$, $P = 0.006$). However, we observed no significant difference in the prevalence of other thyroid diseases among the three areas (Table 2).

Thyroid function, and urinary and serum iodine levels in autoimmune thyroid disease and thyroid peroxidase antibody (–) & thyroglobulin antibody (–) adults in the three areas

Thyroid function, and urinary and serum iodine levels in AITD and TPOAb (–) & TGAb (–) (TPOAb- and TGAb-negative) adults from the three areas are shown in Table 3. Significant sex differences were observed between the AITD group in the ID, IA and IE areas when compared with the TPOAb (–) & TGAb (–) group ($\chi^2 = 21.013$, 5.458 and 6.259; $P \leq 0.001$, 0.019 and 0.012, respectively). The proportion of AITD in the females in each area was higher than males (ID, 27.6 *v.* 6.9%; IA, 14.7 *v.* 6.2%; and IE, 17.3 *v.* 8.1%). There was no significant difference in age between the three areas. However, the FT_3 level in the AITD group in the ID and IE areas was significantly lower than the TPOAb (–) & TGAb (–) group ($Z = -2.467$ and -2.040 ; $P = 0.014$ and 0.041, respectively). Furthermore, the FT_4 level in the AITD group was significantly lower in the IA areas ($Z = -2.028$, $P = 0.043$). Comparatively, the TSH level in the AITD group in each of the three areas was higher than the level in the TPOAb (–) & TGAb (–) group, although these differences were only significant in the ID and IE areas ($Z = -3.621$ and -3.580 ; and all $P < 0.001$, respectively). The BMI, SIC and UIC showed no statistical differences.

Thyroid disease distribution of autoimmune thyroid disease and thyroid peroxidase antibody (–) & thyroglobulin antibody (–) in adults in the three areas

The thyroid disease distribution among the AITD and TPOAb (–) & TGAb (–) adults in the three areas is shown in Table 4. Comparatively, the prevalence of any form of thyroid disease and the total prevalence of thyroid disease among the AITD groups in the three areas did not differ significantly. However, among the TPOAb (–) & TGAb (–) group, the total prevalence

Table 1. Demographic characteristics, urinary iodine, serum iodine and thyroid function in three areas
(Normally distributed mean values and standard deviations; non-normally distributed medians and 25th and 75th percentiles (P25–P75), and percentages)*

Characteristics	ID		IA		IE		P
	Median	P25–P75	Median	P25–P75	Median	P25–P75	
<i>n</i>		409		392		424	
Sex (male/female)		128/281		107/285		138/286	0.239
Age (years)							0.054
Mean		46		45		44	
SD		9		10		11	
BMI (kg/m ²)							0.266
Mean		24.7		24.8		25.4	
SD		3.5		3.6		4.1	
MWI (µg/l)	2.6	1.9–2.8	71.4	18.2–98.2	325.0	300.3–375.2	
Coverage of household use of qualified iodised salt (%)		93.9	–	–	–	–	
UIC (µg/l)	228.4	152.3–314.3	243.9	148.6–358.0	390.4††	247.0–540.3	<0.001
SIC (µg/l)	77.4†	67.6–88.5	74.8	65.4–84.4	81.5††	70.9–90.5	<0.001
FT ₃ (pmol/l)	5.4	5.1–5.8	5.4	5.0–5.7	5.3‡	4.9–5.7	0.001
FT ₄ (pmol/l)	15.7	14.1–17.3	15.9	14.3–17.3	16.8††	15.6–18.3	<0.001
TSH (µIU/ml)	2.0†	1.4–2.8	2.3	1.6–3.1	2.3‡	1.6–3.5	<0.001
TPOAb (+)							0.201
<i>n</i>		45		30		46	
%		11.0		7.7		10.9	
TGAb (+)							<0.001
<i>n</i>		78†		41		48‡	
%		19.1		10.5		11.3	
TPOAb (+) & TGAb (+)							0.379
<i>n</i>		34		23		33	
%		8.1		5.9		7.8	

Iodised salt and thyroid autoimmunity

ID, iodine-deficient areas; IA, iodine-adequate areas; IE, iodine-excess area; MWI, median water iodine; UIC, urinary iodine concentration; SIC, serum iodine concentration; FT₃, free triiodothyronine; FT₄, free thyroxine; TSH, thyroid-stimulating hormone; TPOAb, thyroid peroxidase antibody; TGAb, thyroglobulin antibody; TPOAb (+), thyroid peroxidase antibody-positive; TGAb (+), thyroglobulin antibody-positive; TPOAb (+) & TGAb (+), TPOAb- and TGAb-positive.

* The Kruskal–Wallis test was adopted for UIC, SIC, FT₃, FT₄, TSH; the χ^2 test was used for TPOAb (+), TGAb (+), and TPOAb (+) & TGAb (+). $P < 0.05$ was considered significant.

† ID and IE groups compared with the IA group, respectively. ID group compared with the IA group: SIC ($Z = -2.403$, $P = 0.016$), TSH ($Z = -3.399$, $P = 0.001$), TGAb (+), n (%) ($\chi^2 = 11.735$, $P = 0.001$) and IE group compared with the IA group: UIC ($Z = -9.606$, $P < 0.001$), SIC ($Z = -5.664$, $P < 0.001$), FT₄ ($Z = -6.751$, $P < 0.001$).

‡ IE group compared with the ID group. UIC ($Z = -11.920$, $P < 0.001$), SIC ($Z = -3.194$, $P = 0.001$), FT₃ ($Z = 13.271$, $P < 0.001$), FT₄ ($Z = 74.320$, $P < 0.001$), TSH ($Z = 14.809$, $P < 0.001$), TGAb (+), n (%) ($\chi^2 = 9.740$, $P = 0.002$).



Table 2. Prevalence of thyroid disease among adults in three areas (Numbers and percentages)*

	ID		IA		IE		P
	n	%	n	%	n	%	
n	409		392		424		
Hypothyroxinaemia	2	0.5	5	1.3	2	0.5	0.315
Overt hypothyroidism	4	1.0	2	0.5	1	0.2	0.358
Subclinical hypothyroidism	30	7.3†	47	12.0	69	16.3‡	<0.001
Overt hyperthyroidism	2	0.5	3	0.8	1	0.2	0.540
Subclinical hyperthyroidism	2	0.5	3	0.8	1	0.2	0.540
Autoimmune thyroid disease	89	21.8†	48	12.2	61	14.4‡	0.001
Thyroid nodule	69	16.9	73	18.6	83	19.6	0.594
Goitre	9	2.2	4	1.0	2	0.5	0.063

ID, iodine-deficient areas; IA, iodine-adequate areas; IE, iodine-excess area.
 * The χ^2 test was used for hypothyroxinaemia, overt hypothyroidism, subclinical hypothyroidism, overt hyperthyroidism, subclinical hyperthyroidism, autoimmune thyroid disease, thyroid nodule and goitre. $P < 0.05$ was considered significant.
 † ID, IE groups compared with the IA group, respectively and $P < 0.05$. ID group compared with the IA group, $P < 0.05$. Subclinical hypothyroidism ($\chi^2 = 4.991$, $P = 0.025$) and autoimmune thyroid disease ($\chi^2 = 12.783$, $P < 0.001$).
 ‡ IE group compared with the ID group, $P < 0.05$. Subclinical hypothyroidism ($\chi^2 = 15.853$, $P < 0.001$), autoimmune thyroid disease ($\chi^2 = 7.666$, $P = 0.006$).

of thyroid disease (16.8 %, 95 % CI 12.9 %, 20.7 %) in the IE area was significantly higher than the prevalence in the ID areas (8.1 %, 95 % CI 5.1 %, 11.1 %; $\chi^2 = 11.526$, $P = 0.001$), especially with respect to subclinical hypothyroidism ($\chi^2 = 23.253$, $P < 0.001$), but the prevalence of goitre in the ID areas (1.9 %) was significantly higher than the IA (0.3 %) and IE areas (0.0 %).

The total prevalence of thyroid disease in the AITD group in the ID, IA and IE areas was higher than the TPOAb (–) & TGAb (–) group (ID, $\chi^2 = 25.430$, $P < 0.001$; IA, $\chi^2 = 16.076$, $P < 0.001$; and IE, $\chi^2 = 7.018$, $P = 0.008$). The distribution of thyroid disease in the adults from the three areas in the AITD and TPOAb (–) & TGAb (–) groups is shown in Fig. 2. Thyroid disease in the TPOAb (–) & TGAb (–) group was mainly subclinical hypothyroidism and thyroid nodules; however, more thyroid diseases were observed in the AITD group than the TPOAb (–) & TGAb (–) group.

Relationship between serum and urinary iodine and thyroid function in adults with autoimmune thyroid disease

All subjects with AITD were divided into five groups based on serum iodine levels, as follows: <50, 50–69.9, 70–89.9, 90–109.9 and ≥ 110 µg/l. Non-linear correlations between serum and urinary iodine, as well as FT₃, FT₄ and TSH, were demonstrated. This relationship has been described using scatter plots, as shown in Fig. 3. The levels of urinary iodine and FT₄ increased with increasing median serum iodine values (Fig. 3(a) and (c)), whereas the levels of FT₃ increased, then decreased with increasing levels of the median serum iodine (Fig. 3(b)). The levels of TSH tended to decrease, then increased with increasing levels of the median serum iodine (Fig. 3(d)).

Analysis of the risk factors for autoimmune thyroid disease in adults

The association between the independent variables (sex, age, BMI, education level, family income, MWI, current smoking status, alcohol consumption, UIC, SIC, TSH, goitre, nodules and radiation history) was explored using a univariate logistic

regression model. The results showed that sex, education level, economic income, MWI, cigarette smoking, alcohol consumption, TSH, SIC and frequency of using computers and mobile phones were associated with AITD. Multivariable logistic regression analysis showed that AITD was more likely in females than males (OR = 2.868; 95 % CI 1.411, 5.831; $P = 0.004$). In addition, a MWI ≤ 10 µg/l (iodised salt fortification) (OR = 3.012; 95 % CI 1.920, 4.726; $P < 0.001$), TSH < 0.27 µIU/ml (OR = 5.428; 95 % CI 1.723, 17.096; $P = 0.004$), TSH > 4.2 µIU/ml (OR = 3.176; 95 % CI 2.010, 5.019; $P < 0.001$), SIC > 110 µg/l (OR = 2.228; 95 % CI 1.110, 4.471; $P = 0.024$) and goitre (OR = 6.878; 95 % CI 1.898, 24.930; $P = 0.003$) were independently associated with AITD (Table 5).

Discussion

Iodine is an essential microelement that participates in the synthesis of thyroxine^(1,2). When ingestion is insufficient or in excess of the body's needs, abnormalities can develop. According to the recommendations of WHO/UNICEF/ICCIDD, the median urinary iodine is a good indicator reflecting recent iodine intake and the iodine nutritional status of the population⁽²⁴⁾. In this study, the UIC of adults in the ID, IA and IE areas was 228.4, 243.9 and 390.4 µg/l, respectively. Thus, the iodine status in the ID and IA areas was sufficient, and the iodine status in the IE area was in excess. The results of this study suggested that the USI policy implemented in China with an iodine concentration <10 µg/l significantly improved the iodine nutritional status of adults. However, the serum iodine levels in the three areas were slightly different. The serum iodine levels in the ID and IE areas were higher than the IA areas, and the serum iodine levels in the IE area were the highest. Some studies have shown that people living in areas with different concentrations of water iodine have significantly different urine iodine levels^(34,35). Compared with the urine iodine, serum iodine is an important biomarker of iodine metabolism. It is relatively stable and can truly reflect the body's recent iodine nutrition status; excess iodine is excreted through renal metabolism. The serum iodine

Table 3. Thyroid function, urinary iodine and serum iodine in adults of autoimmune thyroid disease (AITD) and thyroid peroxidase antibody (TPOAb) (–) & thyroglobulin antibody (TGAAb) (–) in three areas (Normally distributed mean values and standard deviations; non-normally distributed medians and 25th and 75th percentiles (P25–P75), and percentages)*

	ID		<i>P</i>	IA		<i>P</i>	IE		<i>P</i>
	AITD	TPOAb (–) & TGAAb (–)†		AITD	TPOAb (–) & TGAAb (–)†		AITD	TPOAb (–) & TGAAb (–)†	
<i>n</i>	89	320		48	344		61	363	
Sex									
Male			<0.001			0.019			0.012
<i>n</i>	8	108		7	106		11	124	
%	6.9	93.1		6.2	93.8		8.1	91.9	
Female									
<i>n</i>	81‡	212		41‡	237		50‡	239	
%	27.6	72.4		14.7	85.3		17.3	82.7	
Age (years)			0.452			0.173			0.867
Mean	45	46		44	46		44	44	
SD	8	9		10	10		10	10	
BMI (kg/m ²)			0.981			0.283			0.670
Mean	24.7	24.7		24.2	24.9		25.4	25.1	
SD	3.3	3.5		3.5	3.9		4.0	4.7	
UIC (µg/l)			0.396			0.784			0.303
Median	220.1	229.6		224.5	243.9		442.0	381.3	
P25–P75	144.0–303.3	154.8–315.9		160.0–389.6	138.2–363.0		240.0–582.0	242.8–523.8	
SIC (µg/l)			0.959			0.460			0.678
Median	77.7	77.5		72.6	75.1		80.0	81.6	
P25–P75	67.5–89.4	67.6–88.6		65.1–83.8	65.4–84.4		72.3–93.1	70.8–90.3	
FT ₃ (pmol/l)			0.014			0.096			0.041
Median	5.3‡	5.4		5.2	5.4		5.2‡	5.3	
P25–P75	4.9–5.7	5.1–5.9		4.9–5.6	5.0–5.8		4.8–5.5	4.9–5.7	
FT ₄ (pmol/l)			0.155			0.043			0.692
Median	15.2	15.7		15.2‡	15.9		16.6	16.8	
P25–P75	13.9–17.0	14.3–17.0		13.5–16.7	14.4–17.3		15.3–18.5	15.6–18.2	
TSH (µIU/ml)			<0.001			0.396			<0.001
Median	2.4‡	1.9		2.4	2.2		3.0‡	2.2	
P25–P75	1.6–4.1	1.3–2.6		1.5–4.3	1.6–3.0		1.9–4.5	1.5–3.3	

Iodised salt and thyroid autoimmunity

ID, iodine-deficient areas; IA, iodine-adequate areas; IE, iodine-excess area; TPOAb (–) & TGAAb (–), TPOAb- and TGAAb-negative; UIC, urinary iodine concentration; SIC, serum iodine concentration; FT₃, free triiodothyronine; FT₄, free thyroxine; TSH, thyroid-stimulating hormone.

* The χ^2 test was used for sex; the independent-samples *t* test was used for age, BMI; the Mann–Whitney *U* test was adopted for UIC, SIC, FT₃, FT₄ and TSH. *P* < 0.05 was considered significant.

† TPOAb (–) & TGAAb (–) group as the control in each area.

‡ AITD group compared with TPOAb (–) & TGAAb (–) group in each area, *P* < 0.05. ID: sex (χ^2 = 21.013, *P* < 0.001), FT₃ (*Z* = –2.467, *P* = 0.014), TSH (*Z* = –3.621, *P* < 0.001); IA: sex (χ^2 = 5.458, *P* = 0.019), FT₄ (*Z* = –2.028, *P* = 0.043); IE: sex (χ^2 = 6.259, *P* = 0.012), FT₃ (*Z* = –2.040, *P* = 0.041), TSH (*Z* = –3.580, *P* < 0.001).

Table 4. Thyroid disease distribution of autoimmune thyroid disease (AITD) and thyroid peroxidase antibody (TPOAb) (–) & thyroglobulin antibody (TGAb) (–) in adults of three areas (Numbers and percentages)*

	ID				IA				IE			
	AITD	%	TPOAb (–) & TGAb (–)	%	AITD	%	TPOAb (–) & TGAb (–)	%	AITD	%	TPOAb (–) & TGAb (–)	%
<i>n</i>	89		320		48		344		61		363	
Hypothyroxinaemia	0	0.0	2	0.6	2	4.2	3	0.9	2	3.3	0	0.0
Overt hypothyroidism	4	4.5	0	0.0	1	2.1	1	0.3	1	1.6	0	0.0
Subclinical hypothyroidism	18	20.2	12†	3.8	12	25.0	35	10.2	13	21.3	53‡	14.6
Overt hyperthyroidism	2	2.2	0	0.0	2	4.2	1	0.3	0	0.0	1	0.3
Subclinical hyperthyroidism	1	1.1	1	0.3	1	2.1	2	0.6	1	1.6	0	0.0
Thyroid nodule	10	11.2	59	18.4	7	14.6	66	19.2	10	16.4	73	20.1
Goitre	3	3.4	6†	1.9	3	6.3	1	0.3	2	3.3	0‡	0.0
Total thyroid disease	25§	28.1	26†	8.1	18§	37.5	49	14.2	19§	31.1	61‡	16.8

ID, iodine-deficient areas; IA, iodine-adequate areas; IE, iodine-excessive area; TPOAb (–) & TGAb (–), TPOAb- and TGAb-negative.

* The χ^2 test was used for hypothyroxinaemia, overt hypothyroidism, subclinical hypothyroxinaemia, overt hyperthyroidism, subclinical hyperthyroidism, thyroid nodule, goitre and total thyroid disease. $P < 0.05$ was considered significant.

† TPOAb (–) & TGAb (–) group in the ID and IE areas compared with the TPOAb (–) & TGAb (–) group in the IA areas, $P < 0.05$. ID areas compared with the IA areas: subclinical hypothyroidism, goitre and total thyroid disease ($\chi^2 = 10.403$, $P = 0.001$; $\chi^2 = 3.989$, $P = 0.046$; $\chi^2 = 6.196$, $P = 0.013$).

‡ TPOAb (–) & TGAb (–) group in the IE area compared with the TPOAb (–) & TGAb (–) group in the ID areas, $P < 0.05$. Subclinical hypothyroidism, goitre and total thyroid disease ($\chi^2 = 23.253$, $P < 0.001$; $\chi^2 = 10.249$, $P = 0.001$; $\chi^2 = 11.526$, $P = 0.001$). The AITD groups of three areas were compared; the prevalence of any form of thyroid disease and the total prevalence of thyroid disease did not differ significantly.

§ AITD group compared with TPOAb (–) & TGAb (–), $P < 0.05$. Total thyroid disease had differed significantly in each area (ID: $\chi^2 = 25.430$, $P < 0.001$; IA: $\chi^2 = 16.076$, $P < 0.001$; IE: $\chi^2 = 7.018$, $P = 0.008$). The TPOAb (–) & TGAb (–) groups of three areas were compared, subclinical hypothyroidism, goitre and total thyroid disease had differ significantly ($\chi^2 = 22.895$, $P < 0.001$; $\chi^2 = 10.000$, $P = 0.007$; $\chi^2 = 11.603$, $P = 0.003$).

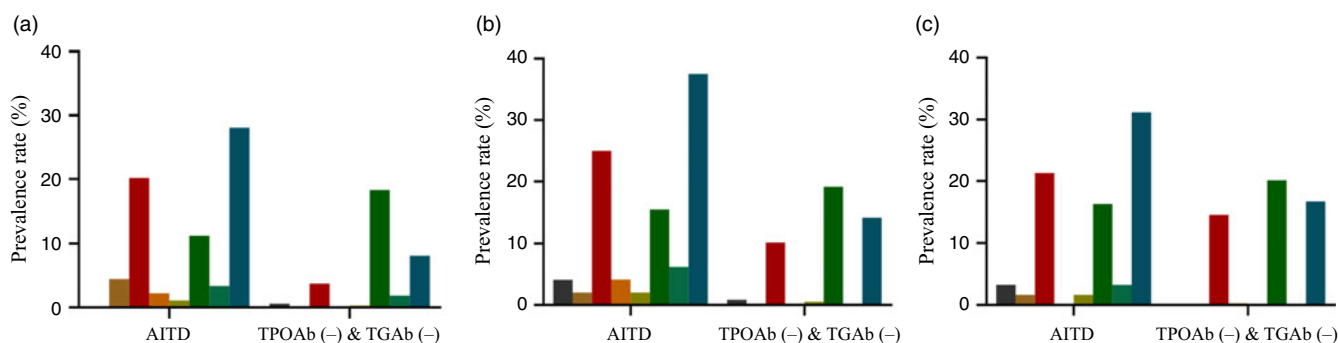


Fig. 2. Thyroid disease distribution of autoimmune thyroid disease (AITD) and thyroid peroxidase antibody (TPOAb) (–) & thyroglobulin antibody (TGAb) (–) in adults of three areas. (a) iodine-deficient areas; (b) iodine-adequate areas; (c) iodine-excessive area. ■, Hypothyroxinaemia; ■, overt hypothyroidism; ■, subclinical hypothyroidism; ■, overt hyperthyroidism; ■, subclinical hyperthyroidism; ■, thyroid nodule; ■, goitre; ■, total thyroid disease. TPOAb (–) & TGAb (–), TPOAb- and TGAb-negative.

level is representative of serum precipitable iodine and is related to thyroid functional status^(36,37). Therefore, the iodine nutrition status of the study population reflected by the serum iodine level may be more stable than urine iodine⁽²⁵⁾. If serum iodine was used to evaluate the iodine nutritional status of the population of this study, the iodine nutritional status in the ID areas was lower than the IE area, but higher than the IA areas.

Animal experiments and demographic epidemiological surveys have shown that excessive iodine intake can lead to abnormal thyroid function, thus, increasing the incidence of thyroid diseases^(35,38). In the present study, the TSH levels of adults in the ID and IE areas were diverse compared with the IA areas. Previous studies have reported that the adult TSH levels increased with an increase in iodine intake^(39,40), which is consistent with the previous data. At the same time, the level of FT₃ in the IE area was lower than the other two areas, while the level of FT₄ was higher, suggesting that high iodine might cause changes

in serum FT₃ and FT₄ levels. In this study, an interesting finding was that although iodine status was sufficient for the ID population, the positive rate of TGAb in ID areas was significantly higher than the other two areas. Although the positive rate of TGAb in the IE area was higher than the IA areas, the positive rate of TGAb was relatively lower than the ID areas, which warrants further study. When analysing the distribution of thyroid diseases in the three areas, it was found that the prevalence of AITD in the ID and IE areas was higher than the IA areas and the prevalence in the ID areas reached 21.8%. Many studies^(13,14) have shown that in ID areas, thyroid-specific autoantibodies (TGAb, TPOAb and TRAb) among residents were higher after iodised salt fortification compared with the levels before iodised salt fortification. Similarly, some non-specific IgA and IgG were also higher than the levels before iodised salt fortification. Nevertheless, there is another issue to consider in this study. Because this study was cross-sectional in design and data on

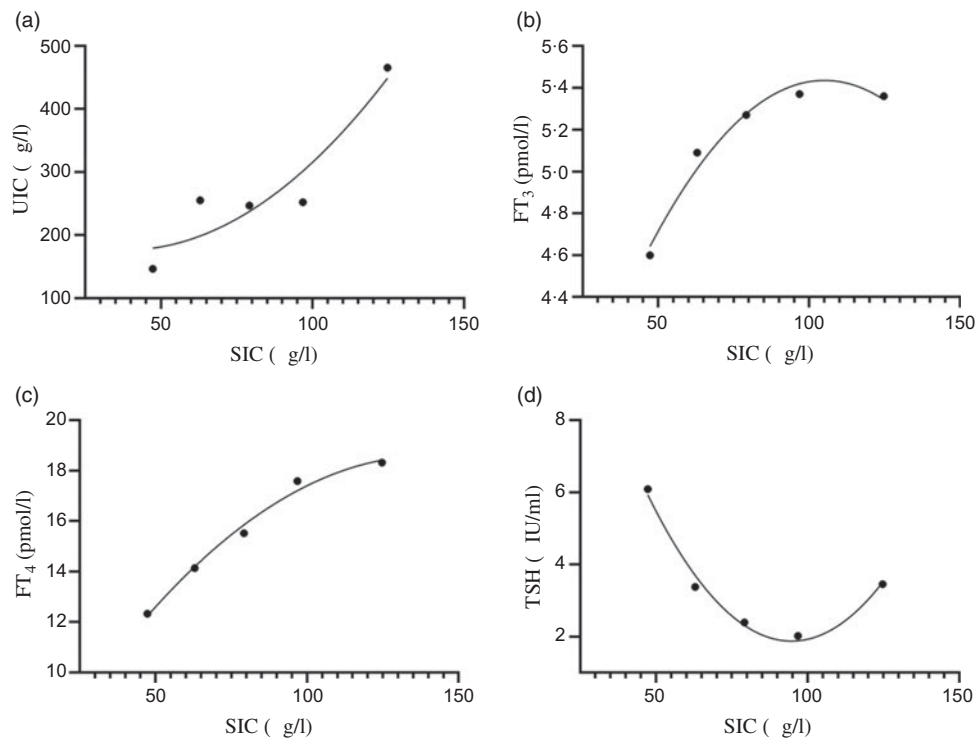


Fig. 3. Relationships between serum iodine and urinary iodine and thyroid function in adults of autoimmune thyroid disease. (a) Serum iodine concentration (SIC) and urinary iodine concentration (UIC); (b) SIC and free triiodothyronine (FT₃); (c) SIC and free thyroxine (FT₄); (d) SIC and thyroid-stimulating hormone (TSH). (a), $R^2 = 0.8700$; (b), $R^2 = 0.9748$; (c), $R^2 = 0.9886$; (d), $R^2 = 0.9849$.

Table 5. Analysis of the risk factors for autoimmune thyroid disease (AITD) in adults (Odds ratios and 95 % confidence intervals)*

Factors	β	Wald	OR†	95 % CI‡	P
Sex					
Male	Ref	Ref	Ref	Ref	Ref
Female	1.054	8.475	2.868	1.411, 5.831	0.004
Water iodine ($\mu\text{g/l}$)					
MWI 40–100	Ref	Ref	Ref	Ref	Ref
MWI ≤ 10 (iodised salt fortification)	1.103	23.012	3.012	1.920, 4.726	<0.001
MWI > 300	0.251	1.061	1.286	0.797, 2.075	0.303
TSH ($\mu\text{IU/ml}$)					
TSH 0.27–4.20	Ref	Ref	Ref	Ref	Ref
TSH < 0.27	1.692	8.351	5.428	1.723, 17.096	0.004
TSH > 4.20	1.156	24.506	3.176	2.010, 5.019	<0.001
SIC ($\mu\text{g/l}$)					
SIC 50–110	Ref	Ref	Ref	Ref	Ref
SIC < 50	0.342	0.396	1.407	0.486, 4.077	0.529
SIC > 110	0.801	5.085	2.228	1.110, 4.471	0.024
Goitre					
No	Ref	Ref	Ref	Ref	Ref
Yes	1.928	8.615	6.878	1.898, 24.930	0.003

Ref, reference; MWI, median water iodine; TSH, thyroid-stimulating hormone; SIC, serum iodine concentration.

* TSH > 4.20 $\mu\text{IU/ml}$ including cases of hypothyroidism, subclinical hypothyroidism; TSH < 0.27 $\mu\text{IU/ml}$ including cases of hyperthyroidism and subclinical hyperthyroidism; goitre, thyroid volume >25 ml (male) and >18 ml (female).

† Model for AITD was adjusted for age, sex and BMI.

‡ Binary logistic regression analysis was used.

thyroid antibody levels in the ID areas were missing before iodised salt consumption, we cannot fully confirm that the higher thyroid antibody levels in the ID areas are related to long-term iodised salt consumption. Nevertheless, the results of this survey are still worthy of our attention. Although the mechanisms by which iodide induces abnormal thyroid antibodies are still

unclear, some scholars believe the following: first, excess iodine induces the production of cytokines and chemokines that can recruit immunocompetent cells to the thyroid; second, processing excess iodine in thyroid epithelial cells may result in elevated levels of oxidative stress, leading to harmful lipid oxidation and thyroid tissue injuries; and finally, iodine incorporation in the



protein chain of thyroglobulin may augment the antigenicity of this molecule⁽⁷⁾. In addition, in this study, we found that the prevalence of subclinical hypothyroidism in IE area was the highest among the three areas studied, which is consistent with the Shan's study and the previous research results of our study team^(6,25,41). No statistical difference was detected among the three groups of other types of thyroid diseases. Our findings provide a reference on the initial dose of iodine used in the implementation of the USI policy in ID areas as an overdose can result in AITD.

According to previous studies, different thyroid antibody status may be related to sex and thyroid hormone levels^(42,43). Therefore, we selected the thyroid antibody double-negative subjects as an internal control of corresponding areas in each area. The findings showed that in each of the three areas, the percentage of AITD in the females was higher than the males (ID, 27.6 *v.* 6.9%; IA, 14.7 *v.* 6.2%; IE, 17.3 *v.* 8.1%), which suggest that independent of the area, women are more likely to have abnormal thyroid antibody levels. This finding is in agreement with a previous study⁽⁴²⁾. In this study, the level of FT₃ in the patients with AITD in the ID and IE areas was significantly lower than the subjects with double-negative antibodies. The level of FT₄ in patients with AITD was significantly lower than the patients with double-negative antibodies in the IA areas. The TSH levels in the AITD group in each of the three areas were higher than the level in the TPOAb (–) & TGAb (–) group, although these differences were only significant among the ID and IE areas. This finding indicates that abnormal thyroid function is more likely to prevail among people with abnormal antibodies than people with double-negative antibodies.

In view of the above results, what is the distribution of thyroid disease in people with different thyroid antibody status in the three areas? We analysed the prevalence of thyroid disease in people with different thyroid antibody status in the three areas to answer this question. The results showed that there was no statistical difference in the total prevalence of thyroid disease among the people with AITD in the three areas and no statistical difference in the prevalence of any form of thyroid disease, suggesting that different water iodine areas had no influence on the types of diseases among the people with AITD. Compared with the TPOAb (–) & TGAb (–) group of the three areas, the prevalence of hypothyroidism (14.6%) in the IE area was higher than the IA (10.2%) and ID areas (3.8%), suggesting that IE is closely related to hypothyroidism, which is consistent with a previous study⁽⁴⁴⁾. In addition, although the prevalence of goitre in the ID areas (1.9%) was higher than the IA (0.3%) and IE areas (0.0%), the proportion was <5% and, therefore, of little practical significance. In this study, we also noted that the total prevalence of thyroid disease in people with AITD was higher than people with TPOAb (–) & TGAb (–) in the three areas, and there were more types of thyroid diseases in the people with abnormal thyroid antibodies in the three areas. Li reported that although some antibody-positive people have normal thyroid function, the probability of thyroid dysfunction is significantly increased⁽⁴⁵⁾. At the same time, people with autoimmune genetic background or positive thyroid autoantibodies are more likely to develop thyroid disease when iodine is sufficient or in excess. The population susceptible to thyroid diseases is appreciable in the general

population, which is estimated to account for approximately 15% of the total population⁽⁴³⁾. Therefore, regular follow-up evaluations are needed for people with abnormal thyroid antibodies, such as finding problems and taking corresponding treatment measures.

Serum iodine is a sensitive indicator used to evaluate individual iodine nutrition status⁽²⁵⁾. Therefore, the level of individual serum iodine may be of great significance for the follow-up evaluation of subclinical and clinical thyroid diseases in people with abnormal thyroid antibody levels. Our results showed that there was a strong non-linear correlation between serum iodine, urine iodine and thyroid function in adults with AITD. We found that urine iodine increased with an increase in serum iodine. Generally, dietary iodine can be detected serially in circulating blood within a few minutes after eating, after which a large proportion of the unabsorbed iodide in blood will be excreted by the kidneys, while the remainder will be taken up by the thyroid and other tissues⁽³⁷⁾. Therefore, iodine concentration rapidly increases in the urine as iodine in the diet is absorbed into the blood⁽³⁶⁾. In addition, some researchers have compared the disappearance rates of iodine from blood with the values obtained from simultaneous observations in urine, finding that the difference was not significant⁽³¹⁾. These results show a strong correlation between serum and urine iodine. Theoretically, both indicators can be used to evaluate the iodine nutrition status of individuals. However, urinary iodine is greatly affected by diet, urine volume, collection time, as well as other factors. Therefore, urine iodine is not an ideal indicator for individual iodine nutrition evaluation. Serum iodine is an important biomarker reflecting recent iodine intake⁽³⁶⁾. To maintain balance, excess iodine can be excreted through the urine⁽⁴⁶⁾. Finally, the iodine deposited in the blood tends to be stable. Therefore, in the evaluation of individual iodine nutrition status, serum iodine may be more sensitive and relatively stable than urinary iodine. It was also found in this study that TSH levels decreased with an increase in serum iodine levels, then increased with an increase in serum iodine levels. The TSH level of AITD adults exhibited a positive 'U' curve relationship with serum iodine levels, suggesting that both high and low serum iodine levels affect thyroid health. In addition, this study also showed that FT₃ and FT₄ levels increase with an increase in serum iodine levels in adults with AITD. We think that thyroid hormone production in the body is insufficient when the serum iodine level is low (<50 µg/l); however, to maintain a normal level of thyroid hormones, the body will promote an increase in the TSH level by a negative feedback mechanism of the thyroid. Subsequently, with an increase in serum iodine levels, thyroid hormone in the body gradually stabilises to meet the normal physiological needs of the human body. Nevertheless, a higher level of serum iodine (≥110 µg/l) may trigger thyroid oxidative stress mechanisms in people who are susceptible to thyroid disease, and the harmful lipid oxidation will cause thyroid tissue damage⁽⁷⁾, such as thyroid follicular cell destruction. In addition, FT₃ and FT₄ are released into the blood, causing serum FT₃ and FT₄ levels to rise. The above findings suggest that people with abnormal thyroid antibodies are more likely to have abnormal thyroid function when serum iodine levels are very low or very high. Serum iodine does hold promise as a biomarker in the follow-up



evaluation of subclinical and clinical thyroid diseases in people with abnormal thyroid antibody levels.

Finally, we also found some evidence related to the risk of AITD in adults. Living in areas where the MWI $\leq 10 \mu\text{g/l}$ with iodised salt fortification increased the risk of AITD, and females were at greater risk than males. This finding is in congruence with a previous study⁽³⁶⁾. Pedersen *et al.*⁽⁴⁶⁾ confirmed that Denmark used to be an area of mild-to-moderate iodine deficiency (median UIC = $61 \mu\text{g/l}$). However, after 5 years of iodine fortification with iodised salt, the iodine status significantly improved (median UIC = $101 \mu\text{g/l}$), but the prevalence of thyroid antibodies increased. TPOAb $>30 \text{ U/ml}$ increased from 14 to 24 % and TGAb $>20 \text{ U/ml}$ increased from 14 to 20 %. In addition, we found that a SIC $> 110 \mu\text{g/l}$ increased the risk of AITD compared with the normal reference range ($52\text{--}109 \mu\text{g/l}$) of serum iodine by inductively coupled plasma-MS (Quest Diagnostics)⁽³²⁾. Notably, this finding was similar to the Jin *et al.* study⁽²⁵⁾. Jin *et al.* believe that SIC $< 50 \mu\text{g/l}$ and $>100 \mu\text{g/l}$ increases the risk of AITD. However, we found no statistical association between serum iodine $<50 \mu\text{g/l}$ and AITD. According to our analysis, the iodine nutrition of adults in the areas we investigated was sufficient or above the recommended level, the level of SIC was basically in the range of the normal reference values, and there were few participants in whom the serum iodine level was $< 50 \mu\text{g/l}$. TSH is also considered to be a risk factor for AITD. TSH increases the risk of AITD if too high or too low. Abnormal TSH levels were divided into two categories in this study: hypothyroidism and subclinical hypothyroidism ($>4.2 \mu\text{IU/ml}$); and hyperthyroidism and subclinical hyperthyroidism ($<0.27 \mu\text{IU/ml}$). Therefore, our results suggest that adults with any of the above thyroid disorders may increase the risk of AITD. Finally, goitre is positively associated with the risk of AITD. Goitre is likely to be accompanied by the occurrence of AITD and should therefore receive attention.

Strengths and limitations

The main advantages of this study are as follows. First, we investigated three different water iodine areas, which provided a better representation of the general population. Second, we found that the thyroid immune response of people in ID areas (iodised salt fortification) was more apparent than IE area, even though many epidemiological investigations have reported that thyroid autoimmune response was more obvious in IE areas^(20,47). We strongly believe that our findings are strengthened by the inclusion of areas with different water iodine levels. Third, we discussed the distribution of thyroid disease in people with AITD and people with double-negative thyroid antibodies in different water iodine levels and clarified the relationship between serum and urine iodine in people with AITD, as well as the relationship between serum iodine and thyroid function in the present study, which were lacking in the other epidemiological surveys. Finally, we discussed the risk factors for AITD in adults. This study also had some limitations. First, due to the limited population with AITD, the study may be biased to some extent; thus, additional cases should be collected for further discussion. Second, adjustments need to be made in matching the sex ratio. In addition, a small number of participants filled out the questionnaire

information with omissions. In the future, field investigations should be strictly controlled.

Conclusion

USI has improved the iodine nutritional status of the population in ID areas in China. However, non-step-by-step iodine fortification may induce the transformation of thyroid autoimmune diseases from recessive-to-dominant in susceptible people. We suggest that a USI policy should be implemented, starting with small doses of iodine in ID areas and gradually reaching the optimal fortification dose of iodine over several stages. In the AITD population of all areas, UIC, FT₃, FT₄ and TSH have a non-linear correlation with serum iodine. The TSH level has a positive 'U' curve relationship with the serum iodine level. An abnormal TSH level, SIC $> 110 \mu\text{g/l}$ and goitre were risk factors for AITD in adults, especially women. People with abnormal thyroid antibodies should have regular follow-up of thyroid function, regular check-ups, and if problems are found, appropriate treatment measures should be offered.

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The contribution of each author is as follows: H. S. and L. L. designed the study; H. S., L. L., S. W., M. Q., H. W., B. R., W. J. and X. W. conducted the research; S. W. analysed the data; S. W. wrote the paper; H. S. and L. L. took primary responsibility for the final content of the manuscript. All authors read and approved the final version of the manuscript.

There are no conflicts of interest to declare.

The authors have nothing to disclose.

References

1. Ma ZF & Skeaff SA (2017) Assessment of population iodine status. In *Iodine Deficiency Disorders and their Elimination*, pp. 15–28 [E Pearce, editor]. Cham: Springer.
2. Eastman C & Zimmermann M (editors) (2018) *The Iodine Deficiency Disorders*. Endotext (Internet). South Dartmouth, MA: MDText.com, Inc.
3. Yadav K & Pandav CS (2018) National iodine deficiency disorders control programme: current status & future strategy. *Indian J Med Res* **148**, 503–510.
4. Leung A & Braverman L (2014) Consequences of excess iodine. *Nat Rev Endocrinol* **10**, 136–142.
5. Shen HM (2012) Current situation and countermeasures of iodine deficiency disorders and iodine excess in China. *Chin J Endemiol* **31**, 239–240. (in Chinese)
6. Liu LX, Wang DD, Liu P, *et al.* (2015) The relationship between iodine nutrition and thyroid disease in lactating women with different iodine intakes. *Br J Nutr* **114**, 1487–1495.

7. Luo Y, Kawashima A, Ishido Y, *et al.* (2014) Iodine excess as an environmental risk factor for autoimmune thyroid disease. *Int J Mol Sci* **15**, 12895–12912.
8. Ren YT, Jia QZ, Zhang XD, *et al.* (2014) Epidemiological investigation on thyroid disease among fertile women in different iodine intake. *Chin J Epidemiol* **35**, 45–48. (in Chinese)
9. Armando F, Lidia M, Norman S, *et al.* (2015) Prevalence of autoimmune thyroiditis and thyroid dysfunction in healthy adult Mexicans with a slightly excessive iodine intake. *Nutr Hosp* **32**, 918–924.
10. ICCIDD (1998) Iodine-induced hyperthyroidism. *ICCIDD Newslett* **14**, 10.
11. Chen ZP & Shu YQ (2000) Iodine causes sexual hyperthyroidism. *Chin J Endocrinol* **19**, 73–75. (in Chinese)
12. Wassie M, Yelland L, Smithers L, *et al.* (2019) Comparison of iodine status pre- and post-mandatory iodine fortification of bread in South Australia: a population study using newborn thyroid-stimulating hormone concentration as a marker. *Public Health Nutr* **22**, 3063–3072.
13. Lind P, Kumnig G, Heinisch M, *et al.* (2002) Iodine supplementation in Austria: methods and results. *Thyroid* **12**, 903–907.
14. Pedersen IB, Knudsen N, Jorgensen T, *et al.* (2003) Thyroid peroxidase and thyroglobulin autoantibodies in a large survey of populations with mild and moderate iodine deficiency. *Clin Endocrinol* **58**, 36–42.
15. Jia QZ, Zhang XD, Shen HM, *et al.* (2016) *Definition and Demarcation of Water-borne Iodine-excess Areas Endemic Areas* (GB/T 19380–2016). Beijing: Chinese Specification Press. (in Chinese)
16. Shen HM, Su XH, Ge XG, *et al.* (2009) *Delimitation for the Endemic Areas of Iodine Deficiency Disorders (IDD)* (GB 16005-2009). Beijing: Chinese Specification Press. (in Chinese)
17. Zhang YX, Chen ZP & Ge PF (2008) *Criteria for the Elimination of Iodine Deficiency Disorders in China* (GB 16006-2008). Beijing: Chinese Specification Press. (in Chinese)
18. Chinese Bureau of Disease Control and Prevention (2019) Survey report on iodine content in drinking water. <http://www.nhc.gov.cn/jkj/s5874/201905/bb1da1f5e47040e8820b9378e6db4bd3.shtml> (accessed November 2019).
19. Jin X, Guan YF, Shen HM, *et al.* (2018) Copy number variation of immune-related genes and their association with iodine in adults with autoimmune thyroid diseases. *Int J Endocrinol* 1705478.
20. Du Y, Gao YH, Meng FG, *et al.* (2014) Iodine deficiency and excess coexist in China and induce thyroid dysfunction and disease: a cross-sectional study. *PLOS ONE* **9**, e111937.
21. Hou CC, Liu ZH, Wang Y, *et al.* (2015) Surveys on iodine nutritional status and thyroid related diseases among adults in areas with different water iodine levels in Tianjin. *Chin Prev Med* **16**, 839–842. (in Chinese)
22. Aday LA & Cornelius LJ (editors) (2006) Deciding how many will be in the sample. In *Designing and Conducting Health Surveys*, pp. 154–193. San Francisco, CA: John Wiley & Sons Inc.
23. Chen CM, Zhao WH, Yang XG, *et al.* (2013) *Criteria of Weight for Adults* (WS/T 428-2013). Beijing: Chinese Specification Press. (in Chinese)
24. WHO/UNICEF/ICCIDD (2007) *Assessment of Iodine Deficiency Disorders and Monitoring Their Elimination, A Guide For Program Managers* (WHO/NDH/01.1), 3rd ed. Geneva: WHO.
25. Jin X, Jiang P, Liu LX, *et al.* (2017) The application of serum iodine in assessing individual iodine status. *Clin Endocrinol (Oxf)* **87**, 807–814.
26. Ma ZF & Skeaff SA (2014) Thyroglobulin as a biomarker of iodine deficiency: a review. *Thyroid* **24**, 1195–1209.
27. Du Y, Gao YH, Feng ZY, *et al.* (2017) Serum thyroglobulin-a sensitive biomarker of iodine nutrition status and affected by thyroid abnormalities and disease in adult populations. *Biomed Environ Sci* **30**, 508–516.
28. National health Commission of the People's Republic of China (2017) Survey Programme of Iodine Content in Drinking Water in China (NO.2017-130). (in Chinese)
29. Wang HY, Liu LJ, Li SH, *et al.* (2007) Study on the method of water iodine detection in areas lacking iodine and high iodine. *Chin J Endocrinol* **26**, 333–336. (in Chinese)
30. Tong YK & Huo JF (2012) *General Test Method in Salt Industry – Determination of Iodine* (GB/T 13025.7-2012). Beijing: National Standard of China. (in Chinese)
31. Zhang YP, Yan YQ, Liu LJ, *et al.* (2016) Method for Determination of Iodine in Urine By As3+ – Ce4+ Catalytic Spectrophotometry (WS/T 107.1-2016). Beijing: Health Standard of China. (in Chinese)
32. Han JH, Wu L, Yu SL, *et al.* (2015) Values of iodine metabolism biomarkers in assessing the iodine nutrition status in surgically treated patients with thyroid disease. *Acta Acad Med Sin* **37**, 221–225. (in Chinese)
33. Weetman A (2004) Autoimmune thyroid disease. *Autoimmunity* **37**, 337–340.
34. Henjum S, Barikmo I, Gjerlaug AK, *et al.* (2010) Endemic goitre and excessive iodine in urine and drinking water among Saharawi refugee children. *Public Health Nutr* **13**, 1472–1477.
35. Teng WP, Shan ZY, Teng XC, *et al.* (2006) Effect of iodine intake on thyroid diseases in China. *N Engl J Med* **354**, 2783–2793.
36. Keating F & Albert A (1949) The metabolism of iodine in man as disclosed with the use of radioiodine. *Recent Prog Horm Res* **4**, 429–481.
37. McCahey W, Keating F & Power M (1949) The behavior of radioiodine in the blood. *J Clin Invest* **28**, 191–198.
38. Calil-Silveira J, Serrano-Nascimento C, Laconca R, *et al.* (2016) Underlying mechanisms of pituitary-thyroid axis function disruption by chronic iodine excess in rats. *Thyroid* **26**, 1488–1498.
39. Laurberg P, Pedersen K, Hreidarsson A, *et al.* (1998) Iodine intake and the pattern of thyroid disorders: a comparative epidemiological study of thyroid abnormalities in the elderly in Iceland and in Jutland, Denmark. *J Clin Endocrinol Metab* **83**, 765–769.
40. Wang WB, Jin Y, Teng WP, *et al.* (2002) Research of epidemiology of serum TSH levels of healthy people in area with different iodine intake in China. *Chin J Endocrinol Metab* **18**, 355–356. (in Chinese)
41. Shan ZY, Teng WP, Li YS, *et al.* (2001) Comparative epidemiologic study on the prevalence of iodine-induced hypothyroidism. *Chin J Endocrinol Metab* **17**, 71–74. (in Chinese)
42. McLeod DS & Cooper DS (2012) The incidence and prevalence of thyroid autoimmunity. *Endocrine* **42**, 252–265.
43. Wang FJ, Guo W & Tian ZH (2018) The relationship between the increase of TGAb or TPOAb antibody and the change of thyroid hormone. *Syst Med* **3**, 15–17. (in Chinese)
44. Teng WP (2010) Iodine nutrition and thyroid disease. *J Intern Med Concepts Pract* **5**, 112–117. (in Chinese)
45. Li YS, Shan ZY, Guan HX, *et al.* (2006) Determination of cut-off points of thyroid peroxidase antibody and thyroglobulin antibody and their clinical significance. *Chin J Lab Med* **29**, 780–783. (in Chinese)
46. Pedersen IB, Knudsen N, Carle A, *et al.* (2011) A cautious iodization programme bringing iodine intake to a low recommended level is associated with an increase in the prevalence of thyroid autoantibodies in the population. *Clin Endocrinol (Oxf)* **75**, 120–126.
47. Li YS, Teng D, Shan ZY, *et al.* (2009) A five-year follow-up study of thyroid peroxidase antibody and thyroglobulin antibody in communities with different iodine intake. *Chin J Intern Med* **48**, 64. (in Chinese).