Thyroglobulin can be a functional biomarker of iodine deficiency, thyroid nodules, and goiter in Chinese pregnant women

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Running title: Elevated Tg increases thyroid nodules prevalence

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ABSTRACT

Background and Objectives: Thyroglobulin (Tg) is considered a sensitive indicator of iodine status for children and adults, but its usefulness for pregnant women is unknown. The aim of this study was attempting to explore the relationship between Tg and iodine status and the association between elevated Tg and thyroid diseases. **Methods and Study Design:** A total of 2163 pregnant women were recruited in this study. The ratio of urine iodine concentration and urine creatinine concentration (UI/Cr) was measured in spot urine samples. Serum thyroid hormones and thyroglobulin were measured. Thyroid nodules and thyroid volume were diagnosed by ultrasound. **Results:** The geometric mean of serum Tg was significantly higher in the UI/Cr <100 μg/g group (10.94 [2.47] μg/L) and the UI/Cr >500 μg/g group (11.48 [2.35] μg/L) than in the 150–249 μg/g group (9.64 [2.32] μg/L). The generalized linear model analysis showed that Serum log(10) Tg concentration was much higher in the UI/Cr <100 μg/g group (β=0.052, *p*=0.026) than in the 150–249 μg/g group. Multivariate logistic regression models demonstrated that elevated Tg may be a risk factor for both goiter (OR=8.30) and thyroid nodules (OR=2.73). **Conclusions:** Pregnant women with UI/Cr <100 μg/g have a higher Tg, and those with elevated Tg concentrations have a higher risk of thyroid nodules and goiter. Tg can be a functional biomarker of iodine deficient, thyroid nodules and goiter.

Key Words: thyroglobulin, iodine status, pregnant women, biomarker, thyroid disease

INTRODUCTION

Iodine is an essential element in the production of thyroid hormones.[1] Given the importance of thyroid hormones in brain maturation, pregnant and lactating women and their fetus and neonates are particularly vulnerable to the effect of iodine deficiency. The recommended dose of iodine for pregnant women in China is 230 μg/day, which is considerably higher than that normally recommended dose for adults (120 μg/day). The iodine requirement in pregnant women is increased due to trans-placental transfer and a 30%–50% increase in renal clearance.[2] Several indicators, such as thyroid goiter rate (TGR), urinary iodine concentration (UIC), and thyroid function, are used to assess the iodine status of a population.[3] The iodine concentration in urine is not a biomarker of thyroid function, and it only reflects the recent iodine intake within the previous 24–48 h.[4] The thyroid volume represents the history of iodine nutrition in a population. However, the TGR may remain high for several years after the introduction of iodized salt, because thyroid volume may not return to normal size in short

term. Thyroid stimulating hormone (TSH) is a sensitive measure of iodine status only in the newborn period.[5]

Tg (thyroglobulin) originates in the thyroid gland and is a precursor in the synthesis of thyroid hormones. It has traditionally been used to monitor the treatment of patients with differentiated thyroid cancer.[6] The serum Tg concentration increases following TSH stimulation and with an increase in the mass of the thyroid tissue.[7] Furthermore, serum Tg values rapidly decrease after iodine supplementation in deficient regions in both children and adults.[8-12] Serum Tg is considered as a promising biomarker of iodine status and reflects thyroid abnormalities and the extent of iodine deficiency in a population.[9,13,14] A multicenter study of 2512 school-aged children from 12 countries with different iodine statuses determined that a median Tg concentration of <13 μg/L or <3% of Tg values >40 g/L can be used as a biomarker of adequate iodine status in children.[15] Additionally, Tg may be used as a functional biomarker of iodine nutrition status in adults.[8,9,16,17]

However, the usefulness of Tg as a biomarker of iodine status in pregnancy is uncertain, because hormonal changes and increased metabolic demands result in complex alterations in thyroid function during pregnancy.[18] Furthermore, conflicting results have been provided regarding the relationship between serum Tg and iodine status in pregnant women.[16,19-22] Therefore, the present study analyzed the relationship between serum Tg concentrations and iodine status during pregnancy. We hypothesized that serum Tg is an effective biomarker of iodine status. In addition, the relationship between elevated Tg levels and thyroid disease was explored in this study.

MATERIALS AND METHODS

The study was conducted from March 2016 to May 2017 and included pregnant women who attended routine antenatal outpatient visits at Tianjin Maternal and Children's Hospital (Tianjin, China), Tanggu Maternal and Children's Hospital, and Zibo Maternal and Child Health Hospital in Gaoqing County. Pregnant women from Tianjin had adequate iodine intake after the implementation of universal salt iodization based on the results of our previous study.[23] Iodine status in pregnant women varies depending on the type of drinking water. The inclusion criteria in the present study were as follows: 1) healthy women aged 20 to 45 years; 2) those who lived for >5 years at their current residence; and 3) no diet restrictions. Participants were excluded if 1) they had occupational exposure to iodine, 2) used thyroid medications or iodine-containing supplements, 3) had a history of thyroid disease, or 4) have the positivity of TPO-Ab of Tg-Ab.

Data collection

All participants were required to complete questionnaires to collect relevant information such as age, gestational week, height, pre-pregnancy and current body weights, history of secondhand smoke exposure, history of thyroid disease, major chronic disease, and use of medications or iodine supplements. The participants were asked to provide 5 mL of a spot urine sample and a blood sample at the obstetric clinic. The serum was separated and stored and the urine samples were stored at −80℃ until further analysis. The pregnant women were required to sit with the neck extended for the ultrasound examination of the thyroid, which was performed by a well-trained radiologist to determine the thyroid volume. The examination was performed using a portable ultrasound diagnostic system (DP-10; Mindray Medical care; Shenzhen; China) equipped with linear array transducers (4 cm, 7.5 MHz). The maximum anteroposterior (D), transverse (W), and longitudinal (L) diameters of the thyroid lobes were recorded on ultrasound images. The sizes were measured to the nearest millimeter. The thyroid volume of each lobe was calculated using the following formula: Tvol (mL) = W × D × L × 0.479/1000. The number of nodules and the maximum diameter of the dominant nodule were recorded.

Laboratory analysis

Spot urine samples were analyzed for the UIC and urine creatinine concentration (UCr) at the Key Laboratory of Hormone and Development (Ministry of Health), Metabolic Diseases Hospital, and the Tianjin Institute of Endocrinology, Tianjin Medical University. UICs were measured using the Sandell–Kolthoff method of quality control,[24] whereas UCr was measured using the spectrophotometric method. In healthy, well-nourished adults, daily creatinine excretion is fairly constant at approximately 1 g; UI/Cr was used to assess the iodine status and to predict 24-h urine iodine excretion.[19] The chemiluminescence immunoassay (Bayer Healthcare of Siemens, Berlin, Germany) was conducted to measure serum Tg, TSH, free thyroxine (FT4), and free triiodothyronine (FT3) levels. Serum TPO-Ab and Tg-Ab titers were measured using the IMMULITE 2000 system (Simens Healthcare Diagnostics Inc, Gowned, United Kingdom).

This study was approved by the Ethics Committee of Tianjin Medical University. All procedures were performed in accordance with the 1964 Helsinki declaration and its amendments or with comparable ethical standards. Informed consent was obtained from all the participants after a full explanation of the study purpose and procedures was provided.

Statistical methods

Data entry and statistical analysis were performed using Excel 2013 and IBM SPSS statistics version 21.0 (IBM Corp. Armonk, NY.USA), respectively. The Kolmogorov–Smirnov test was used to assess data distribution. Normally distributed data are expressed as mean ± standard deviation (SD); the means were compared between two or more subgroups using Student's t-test or one-way ANOVA. Non-normally distributed variables are expressed as the median and interquartile range (IQR), and the Mann–Whitney U test was used to compare differences in means among the trimesters. Tg values were logarithmically transformed to obtain substantial normality before analysis, and geometric means (95% CI) were shown. Differences in $\log_{10}$Tg after adjusting for gestational weeks in different groups were determined using ANCOVA. Categorical variables are expressed as percentages, and proportions were compared using the chi-square test.

The generalized linear model was used to assess the effect of iodine status on log serum Tg values after adjustments for age, gestational week, BMI, thyroid nodules and goiter, secondhand smoke exposure (yes/ no), and region (Tianjin/Gaoqing). Univariate logistic regression and multivariate logistic regression models were constructed to assess the effect of elevated Tg on goiter (>18 mL/≤18 mL) and thyroid nodules (yes/ no), respectively. Two multivariate logistic regression models included the same explanatory variables, such as elevated iodine:creatinine (UI/Cr) ratio, age, gestational week, TSH, pre-pregnancy BMI, secondhand smoke exposure history, and region.

Diagnostic criteria

A serum Tg value of >43.5 μg/L was considered as elevated Tg.[10] Goiter was defined as a thyroid volume of >18 mL. A nodule with a maximum diameter of >3 mm was defined as a thyroid nodule. Iodine nutrition in the pregnant women was divided into five levels based on UI/Cr values: <100 μg/g, 100-149 μg/g, 150-249 μg/g, 250-499 μg/g, and ≥500 μg/g.[20]

RESULTS

A total of 2163 (1613 in Tianjin and 550 in Gaoqing) pregnant women were eligible for the final analysis (Figure 1). The demographics, thyroid function, and urine iodine status of the pregnant women stratified by regions are presented in Table 1. The number of pregnant women in the first, second, and third trimesters was 441 (20.6%), 1135 (53.1%), and 563 (26.3%), respectively. The median Tg of the total participants was 10.9 (6.76, 17.6) μg/L. The median overall UIC and UI/Cr were 171 (113, 266) μg/L and 143 (97, 231) μg/g, respectively.

Significant differences in age, pre-pregnancy weight, current weight, height, and gestational week were observed between different regions. The median UI/Cr was significantly higher (p<0.001) in the pregnant women living in Gaoqing (253 [147, 456] μg/g) than those living in Tianjin (127 [91, 188] μg/g). FT3 and TSH levels were significantly different, whereas no differences in FT4 and Tg were observed between regions. The prevalence of thyroid dysfunction significantly differed between the women in Tianjin and Gaoqing (p=0.04). The pregnant women from Gaoqing (12.7 mL) presented with significantly larger thyroid volumes than those from Tianjin (9.7 mL). Similarly, the prevalence of goiter and thyroid nodules was significantly higher in the women from Gaoqing (p<0.001).

Table 2 displays the pregnancy week–adjusted serum Tg concentration and the percentage of elevated Tg concentration stratified by UI/Cr groups, thyroid nodules, and goiter. Based on UI/Cr values, 572 (26.4%) women were included in the <100 μg/g UI/Cr group, 582 (26.9%) women in the 100-149 μg/g group, 514 (23.8%) women in the 150-249 μg/g group, 340 (15.7%) women in the 250-499 μg/g group, and 155 (7.2%) women in the ≥500 μg/g group. The geometric mean of serum Tg was significantly higher in the <100 μg/g group (10.94 [2.47] μg/L) and the >500 μg/g group (11.48 [2.35] μg/L) than in the 150-249 μg/g group (9.64 [2.32] μg/L), but no significant difference was found among other UI/Cr groups. However, compared with the 150-249 μg/g group, the percentage of elevated Tg was significantly higher in the <100 μg/g group (χ^2 = 8.482, p=0.004).

Figure 2 shows the serum Tg concentrations and results of the Mann–Whitney tests in the goiter and thyroid nodule groups. Significant differences in serum Tg levels were observed between pregnant women with goiter and women in the general population (p=0.046). Serum Tg levels were significantly higher in the women with thyroid nodules than in those without nodules (p < 0.001).

Table 3 shows the results of the generalized linear model analysis; differing iodine intake had substantial effects on log(10)Tg levels. Significant correlations were observed between log(10)Tg levels and FT4 (β=0.014, p=0.002) and gestational week (β=0.003, p=0.001). Serum log(10) Tg levels were much higher in the <100 μg/g UI/Cr group (β=0.052, p=0.026) than in the 150–249 μg/g group.

Table 4 displays the results of the logistic regression models, which tested the relations between thyroid nodules, goiter, and elevated Tg levels. After adjusting for TSH, I/Cr, age, gestational week, pre-pregnancy BMI, and region, elevated Tg levels may be a risk factor for both goiter (OR=8.30) and thyroid nodules (OR=2.73).

DISCUSSION

According to the WHO criteria for UIC, pregnant women in both Gaoqing and Tianjin are iodine sufficient. Furthermore, in the present study, the women living in Gaoqing had higher iodine status than those in Tianjin, which can be explained by the high iodine content in drinking water in Gaoqing County.[25] However, no significant difference was noted in the serum Tg level between the women in Tianjin and Gaoqing. In accordance with previous studies, pregnant women in the <100 μg/g and >500 μg/g UI/Cr groups had higher Tg levels than those in the 150–249 μg/g group.[26-29] Pregnant women with thyroid nodules or goiter had higher serum Tg levels than normal women. Furthermore, we reported independent association of UI/Cr <100 μg/g, FT4 and gestational weeks with serum Tg levels. Elevated Tg were associated with the prevalence of Goiter and thyroid nodules.

Inadequate iodine intake during pregnancy may be detrimental to the fetus, resulting in possible problems in psychomotor development.[30,31] UIC may be an unsuitable marker to assess iodine status during pregnancy because it reflects the iodine intake at a certain time point only. Serum Tg has been used to assess long-term iodine status and existing iodine stores in schoolchildren from different countries[15] and in adults in China.[31] A review conducted by Ma Z et al found that the median Tg in pregnant women from iodine-deficient areas was >13μg/L.[32] However, large observational studies in women with adequate and inadequate iodine status are required to reach a conclusion about the usefulness of Tg as a biomarker of iodine status during pregnancy. The current study included 2163 pregnant and healthy women from Tianjin and Gaoqing with adequate iodine status.

To eliminate the effects of autoimmune destruction of thyroid cells and thyroid dysfunction, the participants who were positive for Tg-Ab or TPO-Ab and those with thyroid dysfunction were excluded from the analysis. In the present study, the pregnant women with iodine:creatinine ratios of <100 μg/g had higher serum Tg concentrations; this result is consistent with the results of another cohort study conducted in the United Kingdom.[21] Insufficient iodine intake induces the a proliferation of the thyroid cells, which leads to an the enhanced turnover of thyroid the cells, resulting in the, and release of Tg into the serum.[33] Additionally, we found that the pregnant women with goiter had significantly higher concentrations of Tg than normal women, and a significant association between the serum Tg concentration and goiter was observed in the logistic regression analysis. However, thyroid volume alone does not explain the elevation of serum Tg. A significant correlation between Tg and FT4 was found in the present study. High Tg levels in the pregnant women with iodine deficiency reflect an increase in thyroid activity to maintain normal thyroid function.[8,9]

In the general linear model, no significant correlation was noted between Tg and TSH, as reported previously.[34,35] This may be due to the upregulation of the synthesis of thyroid hormones and the maintenance of euthyroidism through mechanisms independent of TSH in pregnant women.[36] The increase in the Tg concentration occurs earlier than the increase in the serum TSH level and thyroid volume. Similarly, the decrease in serum Tg occurs earlier than the reduction in the thyroid volume after improvements in iodine nutrition. A study conducted in 1994 proved that the Tg concentration is more sensitive than TSH as an indicator of iodine deficiency.[13] Furthermore, we found that the pregnant women with an UI/Cr of ≥500 μg/g had higher serum Tg concentrations; however, no significant correlation was noted between the serum Tg concentration and ≥500 μg/g UI/Cr was noted. This may be attributed to the small proportion (7.2%) of pregnant women with UI/Cr ≥ 500 μg/g in our study.

The present study showed that higher serum Tg concentrations can reflect iodine deficiency and thyroid activities in pregnant women. This finding is in accordance with the findings of three randomized controlled trials conducted in pregnant women from regions with mild-to-moderate iodine deficiency; these trials have reported that controls had higher Tg concentrations than those who received iodine supplementation.[37-39] However, conflicting reports have been provided for the usefulness of Tg as an indicator of iodine status,[22] because serum thyroglobulin concentrations are increased by hCG during pregnancy.[40] Previous studies have reported significantly higher Tg concentrations in the third trimester in pregnant women than in non-pregnant controls.[21,35,41] This increase in Tg during pregnancy indicates enhanced thyroidal stimulation as a result of reduced iodine intake and not due to the thyrotropic action of hCG, which is decreased in the late stages of pregnancy.[42]

Tg is a promising indicator of iodine deficiency during pregnancy, which carries information on iodine status of long period and on thyroid function. Compared to UIC, Tg shows only little day-to-day variation,[43] and serum Tg had a higher efficacy than thyroid volume in monitoring iodine deficiency in general population.[8] Serum Tg can be used as a monitoring indicator for preventing iodine deficiency during pregnancy, and iodine supplementation can be scientifically performed early to minimize the damage to the offspring's intelligence.

Furthermore, the results of the logistic regression model indicated an association between elevated Tg concentrations and the prevalence of thyroid nodules and goiter, which is consistent with the results of previous studies in adults.[9,16] This suggested that both the structure of the thyroid tissue and the presence of goiter play important roles in thyroid function.

The strengths of this study include the large sample size with wide variation in iodine status. In addition, the creatinine concentration was measured and is expressed as UI/Cr to reduce the variations caused by differences in urine volume.[8] The individual iodine excreted varied considerably during the day, and it is not possible to classify an individual as high or low iodine intake from a casual urine sample.[44] The UI/Cr ratio obtained from the spot urine sample could serve as a useful and reliable alternative to 24-h urine collection.[44-46] In addition, the participants were asked to complete questionnaires for recording their demographic information, and covariates were adjusted to analyze the relationship between Tg and iodine status and thyroid disease. Nevertheless, this study has a number of limitations. First, the cross-sectional design makes it impossible to establish the causal relationship between iodine status and Tg. Second, we did not explore the relationship between Tg and hCG.

In conclusion, our results demonstrated that pregnant women with UI/Cr <100 μg/g have a higher Tg, and those with elevated Tg concentrations have a higher risk of thyroid nodules and goiter. Thus, Serum Tg can be a functional biomarker of iodine deficiency and the presence of thyroid nodules and goiter in pregnant Chinese women.

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CONFLICT OF INTEREST AND FUNDING DISCLOSURE

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Table 1. Characteristics of study participants in different regions

	Tianjin	Gaoqing	*p*	Total
No.	1613	550		2163
Age (years)	28.4±3.7	30.8±4.1	<0.001	29.0±4.1
Gestational week (wk)	21.8±9.3	20.8±8.4	0.020	21.6±9.1
Pre-pregnancy weight (kg)	58.±9.1	59.9±10.2	<0.001	58.8±9.5
Current weight (kg)	62.9±10.3	65.2±10.9	0.092	63.4±10.5
Height (cm)	162.5±4.7	160.6±4.7	0.156	162.1±4.8
UIC (μg/L)	158 (108, 234)	244 (144, 438)	<0.001	171 (113,266)
UI/Cr (μg/g)	127 (91, 188)	253 (147, 456)	<0.001	143 (97, 231)
FT3 (pmol/L)	4.21 (3.88, 4.59)	4.10 (3.82, 4.40)	<0.001	4.18 (3.86, 4.54)
FT4 (pmol/L)	14.65 (13.47, 15.87)	14.57 (13.34, 15.77)	0.269	14.61 (13.42, 15.86)
TSH (mIU/L)	1.51 (0.98, 2.14)	1.75 (1.11, 2.43)	<0.001	1.54 (1.02, 2.22)
Tg (μg/L)	11.0 (6.74, 17.50)	10.90 (6.85, 17.90)	0.588	10.9 (6.76, 17.6)
Tvol (mL)	9.7 (8.0, 11.6)	12.7 (10.2, 16.5)	<0.001	10.2 (8.4, 12.6)
First trimester	308 (19.4%)	133 (24.2%)		441 (20.6%)
Second trimester	846 (53.2%)	289 (52.6%)	0.023	1135 (53.1%)
Third trimester	436 (27.4%)	127 (23.1%)		563 (26.3%)
Goiter (%)	20(1.3%)	81 (15.6%)	<0.001	101 (4.8%)
Thyroid nodules (%)	140 (8.7%)	144(27.4%)	<0.001	284 (13.3%)
Secondhand smoking exposure (%)	122 (11.3%)	52 (9.6%)	0.090	174 (10.8%)
Euthyroidism	1533 (95.1%)	509 (92.6%)		2042 (94.4%)
Hypothyroidism	0 (0.0%)	0 (0.0%)		0 (0.0%)
subclinical hypothyroidism	24 (1.5%)	15 (2.8%)	0.040	39 (1.8%)
Hypothyroxinemia	29 (1.8%)	18 (3.3%)		47 (2.2%)
Thyrotoxicosis	24 (1.5%)	7 (1.3%)		31 (1.4%)

UIC: urine iodine concentration; UI/Cr: iodine/creatinine in spot urine; FT3: free triiodothyronine; FT4, free thyroxine; TSH, thyroid stimulating hormone; Tvol: thyroid volume; Tg: Thyroglobulin.
Data are presented as means±SD, median (IQR) or n (%), with the significance of differences between groups evaluated using t-test, Mann-Whitney rank test or the $\chi 2$ test, respectively.

Table 2. The Tg levels in different iodine status and goiter and nodule group after adjusted gestational weeks

	No.	Tg mean (SD)	$p^{\dagger}$	Elevated Tg (%)	$p^{\ddagger}$
I/Cr group (μg/g)					
<100	572 (26.4%)	10.94 (2.47)	0.012	23 (4.0%)	0.004
100~149	582 (26.9%)	10.23 (2.38)	0.270	13 (2.2%)	0.246
150~249	514 (23.8%)	9.64 (2.32)	Reference	6 (1.2%)	Reference
250~499	340 (15.7%)	9.62 (2.25)	0.955	7 (2.1%)	0.393
>=500	155 (7.2%)	11.48 (2.35)	0.029	6 (3.9%)	0.036
Thyroid volume¶					
Normal	2010 (95.2%)	10.12 (2.35)	0.034	44 (2.2%)	<0.001
Goiter	101 (4.7%)	11.83 (3.01)		10 (9.9%)	
Thyroid nodule§					
Normal	1855 (86.7%)	9.79 (2.38)	<0.001	39 (2.1%)	<0.001
Thyroid nodule	284 (13.3%)	13.24 (2.26)		16 (5.6%)	

UI/Cr: iodine/creatinine in spot urine; Tg: Thyroglobulin.
†Analysis of covariance (ANCOVA) and $p<0.05$ was statistically significant. The analyses were done after logarithmic transformation of serum Tg; results are shown after transforming back to original values.
‡Chi-square tests. $p<0.005$ was statistically significant in I/Cr group, $p<0.05$ was statistically significant in thyroid volume group and thyroid nodule group.
¶Missing 52 values; §Missing 24 values.

Table 3. The generalized linear model analysis for clarifying the determinant factors of log(10) Tg levels

Variables	Single-factor model			Multivariate model		
	β	95% CI	*p*	β	95% CI	*p*
Age	-0.003	-0.007, 0.001	0.174	-0.004	-0.008, 0.000	0.053
Gestational weeks	0.002	0.000, 0.004	0.047	0.003	-0.014, 0.054	0.001
BMI	0.001	-0.005, 0.006	0.822	0.001	-0.005, 0.006	0.824
FT3	0.014	-0.014, 0.042	0.323	0.020	-1.10, 1.12	0.249
FT4	0.011	0.003, 0.019	0.005	0.014	0.005, 0.023	0.002
TSH	0.000	0.000, 0.000	0.922	0.000	0.000, 0.000	0.989
UI/Cr groups						
<100	0.053	0.007, 0.099	0.023	0.052	0.006, 0.098	0.026
100-149	0.013	-0.033, 0.058	0.579	0.011	-0.034, 0.056	0.633
150-249	Reference	-	-	Reference	-	-
250-499	-0.004	-0.057, 0.048	0.871	-0.012	-0.065, 0.042	0.671
≥500	0.072	0.002, 0.142	0.044	0.048	-0.025, 0.120	0.199
Secondhand smoking exposure						
no	Reference	-	-	Reference	-	-
yes	-0.010	-0.070, 0.051	0.753	-0.011	-0.071, 0.048	0.707
Regions						
Tianjin	Reference	-	-	Reference	-	-
Gaoqing	0.027	-0.011, 0.065	0.163	0.019	-0.025, 0.064	0.392

In the single-factor model, only one of the factors were included, whereas in the multivariate model, adjustment was made for the impact of all other variables in this table.

Table 4. Analysis elevated Tg (Tg>43.5μg/L) as a risk factor of Goiter and thyroid nodules

Variables	Unadjusted			Adjusted†		
	OR	95% CI	*p*	OR	95% CI	*p*
Thyroid nodules						
Normal	Reference	-	-	Reference	-	-
Thyroid nodule	2.78	1.53, 5.04	0.001	2.73	1.42, 5.24	0.003
Thyroid volume						
Normal	Reference	-	-	Reference	-	-
Goiter	4.91	2.39, 10.07	0.000	8.30	3.22, 21.38	0.000

Data show adjusted ORs with 95% CIs.
†Logistic regression analysis was adjusted for UI/Cr ratio, age, gestational week, TSH, pre-pregnancy BMI, second smoking exposure history, regions.

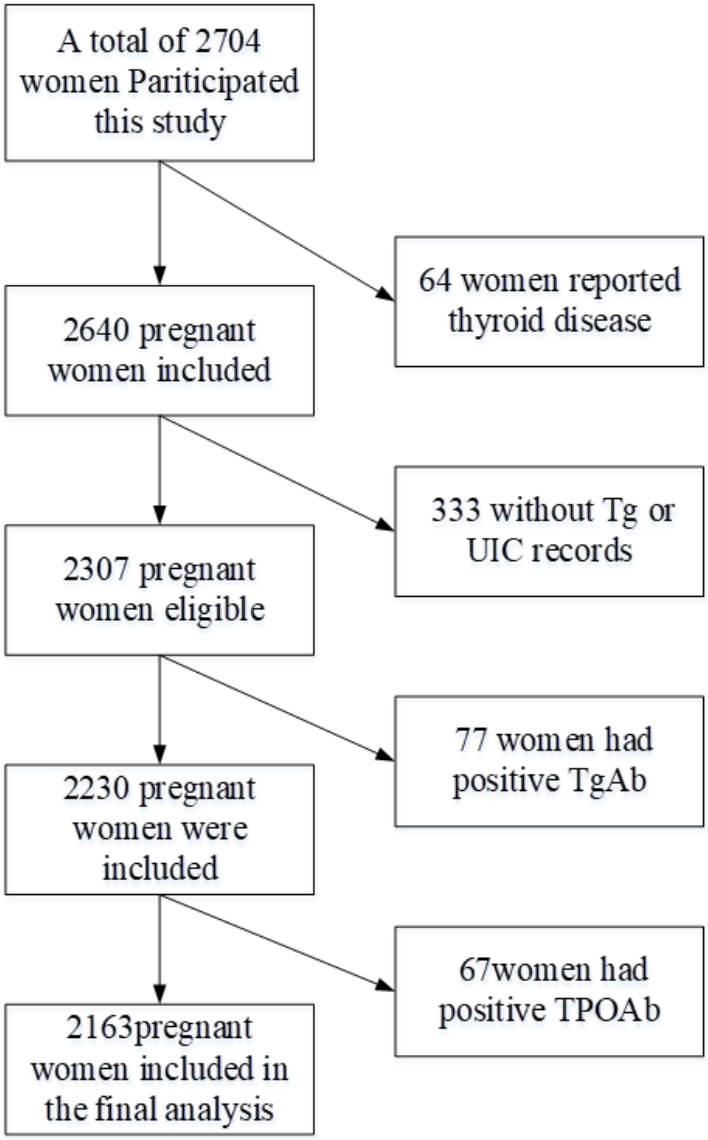

Figure 1. Flowchart of the study population for analysis.

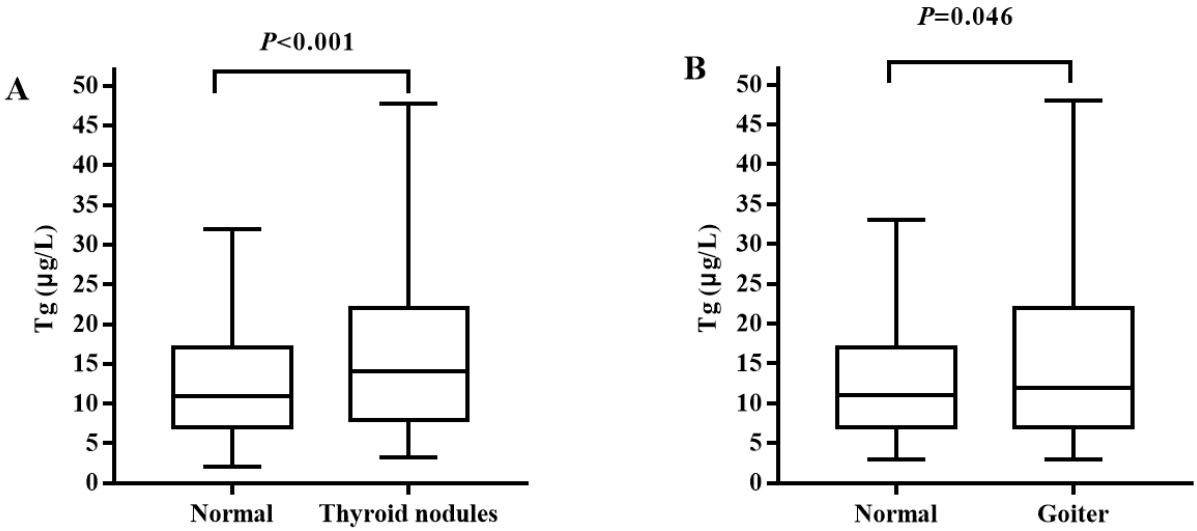

Figure 2. Distribution of Tg among the normal women and those with thyroid nodules and goiter. (A) Box plot showing the distribution of Tg among normal women and those with thyroid nodules. (B) Box plot showing the distribution of Tg among normal women and those with goiter. Upper horizontal line of box, 75th percentile; lower horizontal line of box, 25th percentile; horizontal bar within box, median; upper horizontal bar outside box, 95th percentile; lower horizontal bar outside box, 5th percentile.