

Iron Deficiency May Predict Greater Risk for Hypothyroxinemia: A Retrospective Cohort Study of Pregnant Women in China

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Abstract

Background

Pregnant women are highly vulnerable to iron deficiency (ID) due to the increased iron needs during pregnancy. ID decreases circulating thyroid hormone concentrations likely through impairment of iron-dependent thyroid peroxidase (TPO). The present study aimed at exploring the association between ID and hypothyroxinemia in a retrospective cohort of pregnant women in China.

Methods

To investigate the relationship between ID and hypothyroxinemia, we retrospectively analyzed 723 pregnant women in the present study, including 675 and 309 women in the second trimester and third trimester, respectively. The trimester-specific hypothyroxinemia was defined as free thyroxine (FT₄) levels below the 2.5th percentile of the reference range with serum thyrotropin (TSH) levels in the normal or higher than the 97.5th percentile of reference range in each trimester of pregnancy. Serum TSH, FT₄, thyroid peroxidase antibodies (TPOAb), thyroglobulin antibodies (TgAb), serum ferritin (SF), soluble transferrin receptor (sTfR), and urinary iodine concentrations (UIC) were measured. SF, sTfR, and total body iron (TBI) were used to indicate the nutritional iron status.

Results

Cross-sectional multiple linear regression analysis showed that iron status was positively associated with serum FT₄ levels in the first and second trimesters of pregnancy, but not in the third trimester. Logistic regression analysis showed that ID was an independent risk factor for hypothyroxinemia (odds ratio [OR] = 14.86, 95% confidence interval [95% CI] = 2.31-95.81, $p = 0.005$ in the first trimester and OR = 3.36, 95% CI = 1.01-11.21, $p = 0.048$ in the second trimester). The prospective analysis showed that pregnant women with ID during the first trimester of pregnancy had lower serum FT₄ levels and a higher rate of hypothyroxinemia in the second or third trimester than those without ID.

Conclusions

ID appears to be a risk factor to predict hypothyroxinemia in the first and second trimesters of pregnancy, but not in the third trimester. We suggest that pregnant women with ID in the first and second trimesters be regarded as a high-risk group for maternal hypothyroxinemia.

Introduction

Iron deficiency (ID) is the most common nutritional deficiency worldwide and the leading cause of anemia [1]. The World Health Organization (WHO) estimates that 23% of pregnant women in industrialized countries and 52% in non-industrialized nations suffer from anemia, mostly due to ID, indicating that pregnant women are particularly vulnerable to ID [2-3].

In recent years, the impact of thyroid hormone deficiency on pregnancy outcomes and offspring intelligence has developed into an important and controversial area in the field of endocrinology [4]. Both perinatal thyroid insufficiency and ID are associated with an increased risk of adverse pregnancy complications and impaired fetal neurocognitive development [5-7]. Many studies have shown that ID can reduce thyroid hormone synthesis by having a negative impact on the iron-dependent thyroid peroxidase (TPO), an essential enzyme in the biosynthesis of thyroid hormones [8]. In addition to that, ID was suspected to modulate thyroid metabolism by attenuating oxygen transport, a similar result in hypoxia [9-10]. In 2007, Zimmermann *et al.* first reported that ID was related to lower T₄ during pregnancy in a cross-sectional study of women living in an area with borderline iodine deficiency [11].

The Subclinical Hypothyroid in Early Pregnancy (SHEP) Study, aiming to evaluate thyroid insufficiency on maternal health or infant outcomes, was conducted in Liaoning Province of China between 2012 and 2015. Nineteen hospitals were involved in this study, including the Department of Obstetrics and Gynecology, and the Department of Endocrinology [5,12]. A total of 12,236 women were enrolled in the SHEP study until September 2015, including 9964 pregnant women in the first trimester and 2272 non-pregnant women of childbearing age. Among the 9964 pregnant women, 1834 were followed in the second trimester (between 13 and 28 weeks of gestation) and/or the third trimester (between 29 and 40 weeks of gestation) of pregnancy. Our previous cross-sectional study found a positive relationship between ID and hypothyroxinemia in pregnant women during their first trimester and non-pregnant women of childbearing age [13], however, no causality between ID and hypothyroxinemia was demonstrated. To

address the effects of ID on thyroid hormone levels during pregnancy, we conducted a project that used surplus sera to measure soluble transferrin receptor (sTfR), serum ferritin (SF), and total body iron (TBI) in pregnant women who were included in the SHEP study. The present study aimed to investigate whether ID during an earlier trimester of pregnancy is associated with lower serum free thyroxine (FT₄) levels and an increased risk for hypothyroxinemia in a later trimester of pregnancy.

Materials and Methods

Subjects

The SHEP study aiming to evaluate the impact of thyroid insufficiency on maternal health or infant outcomes was conducted between 2012 and 2015 in Liaoning Province of China, a region where iodine status was adequate [12]. Nineteen hospitals were involved in this study, including the Department of Obstetrics and Gynecology, and the Department of Endocrinology. To be enrolled in the SHEP study, women had to be residents in the local area for more than ten years, aged 19 to 40 years, and planned to become pregnant or had a singleton pregnancy at 4 to 12 weeks of gestation. In addition to that, they did not have a history of thyroid disease or any other chronic diseases and did not take any oral contraceptives or medical regimen that may affect thyroid function, such as glucocorticoids, dopamine, as well as antiepileptic drugs [12]. Basic clinical information was obtained from all the participants, including personal information, personal and family history of thyroid diseases, parity, smoking or drinking, chronic diseases, and multiple-micronutrient supplementation. The height and weight of each participant were measured, their thyroid was palpated, and fasting serum and urine samples were collected. By 2015, 9964 pregnant women were recruited and had blood drawn to test their thyroid function, but only 1834 of them participated in a follow-up study during pregnancy. In the present study, we retrospectively analyzed the data from these 1834 pregnant women, as well as 2272 non-pregnant women of childbearing age from the SHEP study. After excluding the participants with self-reported blood diseases, infections, fever, drug treatments, supplementation treatments (iron, iodine, L-T₄ or anti-thyroid medication), as well as positive thyroid autoantibodies (TPOAb, TgAb), a total of 723

pregnant women and 1645 non-pregnant women were selected. These final participants had blood drawn to measure their levels of serum ferritin (SF) and serum soluble transferrin receptor (sTfR). Among the 723 pregnant women, 675 and 309 women participated in the second trimester and third trimester, respectively, and 261 pregnant women followed in both the second and third trimesters. The flow diagram for the selected subjects was described in Figure 1.

Methods

Samples of urine and blood were obtained from each participant after an overnight fast. All specimens were frozen at -20°C until shipment, then transferred on dry ice to the Endocrinology Institute of China Medical University, and assayed within one week.

Serum thyrotropin (TSH), free thyroxine (FT₄), TPOAb, thyroglobulin antibodies (TgAb), and SF were measured using electrochemiluminescence immunoassays with a Cobas Elecsys 601 platform (Roche Diagnostics, Switzerland). The intra-assay coefficients of variation for serum TSH, FT₄, TPOAb, TgAb, and SF were 1.57-4.12%, 2.24-6.33%, 2.42-5.63%, 1.3-4.9%, and 1.43-4.52%, respectively; the inter-assay coefficients of variation were 1.26-5.76%, 4.53-8.23%, 5.23-8.16%, 2.1-6.9%, and 3.52-7.91%, respectively [12-13]. Serum sTfR was measured using the immunoturbidimetric assay on a Cobas C501 analyzer (Roche Diagnostics, Switzerland). The intra- and inter-assay coefficients of variation for sTfR were 2.26-5.46% and 3.57-6.24%, respectively [13]. Urinary iodine concentrations (UIC) were determined by the ammonium persulfate method based on the Sandell-Kolthoff reaction. The intra- and inter-assay coefficients of variation for the UIC was 3-4% and 4-6% at 66 µg/L and 2-5% and 3-6% at 230 µg/L, respectively [13].

Diagnostic criteria for iron deficiency (ID) were defined as SF < 12 µg/L [14-15], or sTfR > 4.4 mg/L [14], or total body iron (TBI) < 0 mg/kg [13-14]. TBI was calculated from sTfR and SF concentrations with a formula from Cook *et al.* [16-18], as previously described by Yu *et al.* [13]. Positive values of TBI indicated adequacy of iron storage, and negative values indicated ID [11,13,17].

TSH and FT₄ were 0.27-4.2 IU/mL and 12-22 pmol/L, respectively, according to the manufacturer's reference ranges. In the present study, hypothyroxinemia included both

isolated hypothyroxinemia (IH) and overt hypothyroidism (OH). The trimester-specific IH was defined as FT₄ levels below the 2.5th percentile of reference range with normal TSH levels (P2.5th - P97.5th percentiles) in each trimester of pregnancy. The trimester-specific OH was defined as FT₄ levels below the 2.5th percentile with TSH levels higher than the 97.5th percentile of the reference range in each trimester of pregnancy, and subclinical hypothyroidism as TSH levels higher than the 97.5th percentile of reference range with normal FT₄ levels (P2.5th - P97.5th percentiles) in each trimester of pregnancy. The first trimester-specific reference ranges for TSH and FT₄ were 0.14-4.87 mIU/L and 12.35-20.71 pmol/L, respectively, as previously described by Li *et al.* [12]. The reference population, including 986 pregnant women in the second trimester and 469 pregnant women in the third trimester, of the second and third trimester-specific reference ranges for serum FT₄ and TSH was selected according to Guideline 22 developed by the National Academy of Clinical Biochemistry [19]. Thus the second trimester-specific reference ranges for TSH and FT₄ were 0.36-4.42 mIU/L and 9.08-16.89 pmol/L, respectively. Also, the third trimester-specific reference ranges for TSH and FT₄ were 0.51-3.79 mIU/L and 8.40-13.81 pmol/L, respectively. The manufacturer's reference ranges for SF, TPOAb, TgAb, and sTfR were 15-150 µg/L, 0-34 IU/mL, 0-115 IU/mL, and 1.9-4.4 mg/L, respectively.

Statistical analysis

All statistical analyses were performed using SPSS version 21.0 software. The Kolmogorov-Smirnov method was used to test the data distribution. The data following normal distribution are presented as mean ± SDs, and an independent sample *t*-test was used to assess the difference between two groups. The other data are presented as median, and the Kruskal-Wallis *H* test of ranked groups was utilized. Enumeration data are presented as percentages (cases), tested by Chi-square (χ^2) test. Spearman's rank correlation analysis was used to examine the correlation between FT₄ and age, body mass index (BMI), TSH, UIC, SF, sTfR, and TBI. Multiple linear regression analysis was used to test the association between FT₄ and its related risk factors. Multiple logistic regression analysis was used to analyze the risk factors of hypothyroxinemia further. *P* values < 0.05 were considered as statistically significant.

Ethics committee approval

The experimental procedure described was approved by the Ethics Committee of China Medical University ([2012]2011-32-4), and was congruent with the Declaration of Helsinki. Written informed consent was obtained from every participant.

Results

(1) Both iron indicators and thyroid hormone levels show a similar tendency during different trimesters of pregnancy.

The non-pregnant women of childbearing age had similar age and body mass index (BMI) as the pregnant women in the first trimester (Table 1). The serum levels of iron indicators (SF, sTfR, TBI) and FT₄ were compared between the non-pregnant women and pregnant women in the different trimesters of pregnancy (Table 1). In the first trimester, pregnant women had lower sTfR levels and higher TBI, SF, and FT₄ levels compared to non-pregnant women. As the pregnancy progressed, serum TBI, SF, and FT₄ decreased while sTfR increased, indicating a gradual decline of iron and thyroid hormone levels during pregnancy.

(2) Cross-sectional data analysis: ID during the first and second trimesters of pregnancy is associated with lower serum FT₄ levels and an increased rate of hypothyroxinemia during this same period, but this association is not maintained during the third trimester.

First trimester:

According to the trimester-specific reference ranges for serum FT₄ and thyrotropin (TSH), thirteen subjects were diagnosed with hypothyroxinemia in the first trimester. The pregnant women with ID had lower serum FT₄ levels and a higher rate of hypothyroxinemia relative to those without ID in the first trimester (Table 2). The cross-sectional correlation analysis showed a correlation between some independent variables and serum FT₄. SF ($r = 0.12$, $p = 0.001$) and TBI ($r = 0.129$, $p < 0.001$) were positively associated with serum FT₄ levels. BMI ($r = -0.211$, $p < 0.001$), sTfR ($r = -0.076$, $p = 0.04$), and urinary iodine ($r = -0.077$, $p = 0.04$) were negatively associated with serum FT₄ levels. Further, multiple linear regression analysis showed that serum TBI, BMI, and urinary iodine

were associated with serum FT₄ levels (Table 3). To analyze the risk factors for hypothyroxinemia, we applied multiple logistic regression analysis using maternal age, gestational week, ID, BMI, and urinary iodine as independent variables, and hypothyroxinemia as the dependent variable. We found that ID was an independent risk factor for hypothyroxinemia (odds ratio [OR] =14.86, 95% confidence interval [95% CI] = 2.31-95.81, $p = 0.005$) (Table 4).

Second trimester:

Twenty subjects were diagnosed with hypothyroxinemia in the second trimester according to the trimester-specific reference ranges for serum FT₄ and TSH. Similar to the first trimester, the pregnant women with ID had lower serum FT₄ levels and a higher rate of hypothyroxinemia compared with those without ID in the second trimester (Table 2). Serum FT₄ levels were positively correlated with serum SF ($r = 0.211$, $p < 0.001$) and TBI ($r = 0.255$, $p < 0.001$), but negatively associated with maternal age ($r = -0.176$, $p < 0.001$), gestational week ($r = -0.52$, $p < 0.001$), and sTfR ($r = -0.258$, $p < 0.001$). Further, the association of serum FT₄ levels with serum TBI, gestational week, and maternal age was revealed by multiple linear regression analysis (Table 3). Multiple logistic regression analysis was performed to analyze the risk factors for hypothyroxinemia using maternal age, gestational week, and ID as independent variables, and hypothyroxinemia as the dependent variable. In this analysis, we also found that ID was an independent risk factor for hypothyroxinemia (OR = 3.36, 95% CI = 1.01-11.21, $p = 0.048$) (Table 4).

Third trimester:

According to the trimester-specific reference ranges for serum FT₄ and TSH, nine subjects were diagnosed with hypothyroxinemia in the third trimester. There was no significant difference in either serum FT₄ levels or the rate of hypothyroxinemia between the pregnant women with or without ID in the third trimester (Table 2). Univariate analysis showed that urinary iodine was negatively associated with serum FT₄ levels ($r = -0.077$, $p = 0.04$). Multiple linear regression analysis also showed that urinary iodine was negatively associated with serum FT₄ levels (Table 3). However, no risk factor for hypothyroxinemia was apparent (Table 4).

(3) Prospective data analysis: ID during the first trimester of pregnancy is associated with lower serum FT₄ levels and an increased rate of hypothyroxinemia in the second or third trimester of pregnancy

The cross-sectional data from both first and second trimesters in the pregnant women showed that iron stores were positively correlated with maternal serum FT₄ levels. We further studied prospectively whether the iron status in an earlier trimester of pregnancy would affect serum FT₄ levels or the rate of hypothyroxinemia in a later trimester of pregnancy.

Firstly, we compared the serum FT₄ levels of the second or third trimester between women with or without ID in the previous trimester (Supplemental Table 1-3). Compared to the women without ID, the women with ID in the first trimester had lower serum FT₄ levels in both second (Supplemental Table 1) and third (Supplemental Table 2) trimesters. However, no significant difference was found in the serum FT₄ levels of the third trimester between the pregnant women previously diagnosed with or without ID in the second trimester (Supplemental Table 3).

Secondly, we analyzed the rate of hypothyroxinemia of the second or third trimester in pregnant women who were diagnosed with or without ID in their previous trimester. The results showed that the pregnant women diagnosed with ID in the first trimester had a higher rate of hypothyroxinemia in the second and third trimesters compared with those without ID. However, the difference was not statistically significant, which might be mainly due to the limited numbers of participants with ID in the first trimester (Supplemental Tables).

Discussion

The association between iron stores and thyroid hormone has been recently identified. Our previous study reported a positive correlation between ID and hypothyroxinemia during the first trimester of pregnancy [13]. In this present study, we retrospectively studied 723 pregnant women from the SHEP study throughout their entire pregnancies, providing us the opportunity to analyze the association of ID with hypothyroxinemia within each trimester of pregnancy. The data reveal that in an area without iodine deficiency iron

status was positively associated with serum FT₄ levels in the first and second trimesters of pregnancy, but not in the third trimester. In addition to this, our study also shows that pregnant women with ID in the first trimester had lower serum FT₄ levels and a higher rate of hypothyroxinemia in the second or third trimester compared to those without ID.

Overt hypothyroidism (OH) and isolated hypothyroxinemia (IH) in pregnancy have been shown to be associated with an increased risk of both adverse pregnancy complications and poor fetal neurocognitive development [20-23]. Currently, a case-finding approach to identify women at high risk of thyroid dysfunction during pregnancy is recommended. According to our study, we suggest that pregnant women with ID in the first and second trimesters be regarded as a high-risk group for maternal hypothyroxinemia.

ID is the most prevalent micronutrient deficiency worldwide and is a public health problem in both industrialized and non-industrialized countries. Pregnant women and women of childbearing age are at high risk of ID [1]. It is estimated that almost 50% of women do not have adequate iron stores for pregnancy [24-25]. Even in Europe, nearly 20% of women of childbearing age do not have adequate iron reserves [26]. However, it is still controversial whether or not physicians should recommend universal screening for ID anemia in pregnant women to prevent adverse maternal health and birth outcomes [27-30]. Pregnant women are usually advised to take iron supplements to avoid ID anemia during the second half of pregnancy, which is a period of rapid fetal growth and development [1, 27, 31]. Maternal thyroid hormones play an essential role in the neurologic development of the fetus during the first half of pregnancy, because the fetus itself does not produce thyroid hormones until 18-20 weeks of gestation [31-32]. Prospective studies have also shown that thyroid deficiency in the first half of pregnancy can adversely affect neurodevelopment of the offspring [21, 22, 33]. Our previous work has indicated that ID first impairs the iron-dependent TPO activity, an essential enzyme in thyroid hormone synthesis, followed by decreasing the iron content in fetal brain, as the maternal iron is more critical for fetal brain development [34]. Therefore, perinatal ID can lead to maternal and neonatal hypothyroxinemia, which impairs early brain development before the perinatal brain iron depletion, indicating that hypothyroxinemia, instead of perinatal brain iron depletion, might be a primary mechanism underlying impairment of

brain development [35]. However, until now there has been no evidence that treatment of ID reverses hypothyroxinemia in the first or second trimester of pregnancy.

An unusual finding of this study was that ID was associated with an increased risk for hypothyroxinemia in both the first and second trimesters of pregnancy, but not in the third trimester. We propose two possible explanations for this finding. First, the decrease of FT₄ levels due to the poor performance of immunoassays in the third trimester may cause the change in an association with ID [36]. Second, the physiologic hemodilution during the third trimester may also contribute to the lower FT₄ levels [37]. During the second half of pregnancy or the third trimester of pregnancy, it is estimated that the total blood volume increases by almost 45%, with an increase of nearly 50% in plasma volume and an increase of nearly 35% in red blood cell mass [31,38]. The disproportionate increase between plasma volume and red blood cell mass during pregnancy leads to the decreases in hemoglobin and SF concentrations, making it difficult to distinguish physiologic hemodilution from ID during the third trimester [24]. Scholl *et al.* previously reported that ID anemia during the first two trimesters of pregnancy was associated with a two-fold increased risk for preterm delivery and a three-fold increased risk for delivering a baby with low birth weight [39]. However, it has been reported that ID anemia during the third trimester was associated with a reduced risk for preterm delivery and low birth weight; some studies even demonstrated an absent correlation between ID anemia in the third trimester and preterm birth [40-41]. In the present study, as the pregnancy progressed, SF and TBI gradually decreased, suggesting the occurrence of both hemodilution and iron mobilization from body stores to meet the increased demands associated with pregnancy [31]. In contrast, sTfR concentrations were reduced in the first two trimesters of pregnancy and increased in the third trimester, consistent with the increased erythropoiesis observed in the third trimester [42-43].

The present study focused on investigating the effect of ID on hypothyroxinemia. However, there has been only limited evidence about the relationship between thyroid function and iron metabolism. Hepcidin, a liver-derived peptide hormone, is one of the most important regulators of iron homeostasis. Recently, Fischli *et al.* reported that hepcidin and SF levels were higher in patients with Graves' hyperthyroidism when

compared to patients with euthyroidism [44]. Subsequent experiments demonstrated that T_3 increased hepcidin mRNA expression in HepG2 cells [44]. T_3 was also found to up-regulate *ferritin* gene expression through the modulation of iron regulatory proteins (IRPs) binding activities and enhancement of IRE-dependent translation [45]. Unfortunately, FT_3 and hepcidin levels were not measured in the present study. Further evidence is required to elucidate whether maternal hypothyroxinemia leads to decreased levels of hepcidin or SF that results in the reduction of iron reserves.

In order to examine the hypothesis whether ID is a risk factor for hypothyroxinemia, we carefully designed analyses in this study. Firstly, to reduce the interference in studying the association between iron and thyroid hormone, this study excluded pregnant women who had taken any drug or supplements containing iron or iodine during pregnancy, as well as women who had received L-thyroxine ($L-T_4$) treatment or anti-thyroid drugs. Secondly, the present study also excluded pregnant women with positive thyroid autoantibodies, which are known to have an increased risk of hypothyroxinemia.

It should be mentioned that there are several limitations in the present study. Firstly, pregnant women in the first trimester with a history of chronic diseases, including ID anemia, were excluded, likely underestimating the effects of ID on thyroid hormone levels during the period of pregnancy. Secondly, the 723 pregnant women analyzed in the present study were not randomly enrolled resulting in fewer cases of subclinical hypothyroidism and IH in the first trimester, possibly introducing selection bias. Thirdly, the sample size in the present study is relatively small; thus the relationship between ID and IH or OH could not be analyzed separately. Therefore, larger prospective trials to replicate these findings are needed in the future.

In conclusion, for the first time, we report that ID is associated with an increased rate of hypothyroxinemia during the first and second trimesters of pregnancy. We suggest that pregnant women with ID in the first and second trimesters should be regarded as a high-risk group for maternal hypothyroxinemia.

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Author Disclosure Statement

The authors declare no competing or financial interests.

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Table 1. Demographic characteristics, thyroid and iron indicators in the pregnant women and non-pregnant**women of childbearing age in different trimesters of pregnancy**

	Non-pregnancy (n = 1645)	First Trimester (n = 723)	Second Trimester (n = 675)	Third Trimester (n = 309)	P
General					
Age (years)	29.05 ± 3.75	29.29 ± 3.49			
BMI (kg/m ²)	21.86 ± 3.48	21.96 ± 3.15			
Smoking (%)	1.03 (17/1645)	0.69 (5/723)			
Drinking (%)	2.31 (38/1645)	2.62 (19/723)			
GW (weeks)		8.02 ± 1.16	20.21 ± 2.96	30.16 ± 0.98	
Laboratory tests					
TSH (mIU/liter)	2.07 (1.51-2.86) ^a	1.50 (1.02-2.25) ^b	1.62 (1.16-2.32) ^b	1.68 (1.17-2.24) ^b	< 0.001
FT4 (pmol/liter)	15.69 ± 2.40 ^a	16.25 ± 1.96 ^b	12.50 ± 2.17 ^c	11.00 ± 1.57 ^d	< 0.001
SF (µg/liter)	51.27 (29.15-80.22) ^a	66.53 (41.18-100.80) ^b	41.34 (24.90-67.24) ^c	14.26 (9.29-26.16) ^d	< 0.001
sTfR (mg/liter)	3.11 (2.60-3.75) ^a	2.58 (2.19-3.13) ^b	2.44 (2.08-2.86) ^c	3.60 (2.99-4.44) ^d	< 0.001
TBI (mg/kg)	6.81 (4.63-8.66) ^a	8.51 (6.39-10.28) ^b	6.97 (4.73-8.92) ^a	1.65 (-0.27-4.54) ^c	< 0.001
UIC (µg/liter)	164.30 (100.09-	170.22 (124.57-	138.33 (89.58-	130.46 (85.08-198.19	< 0.001
Prevalence of thyroid diseases					
Overt hypothyroidism	0.12% (2)	0%	0.74% (5)	0	0.05
Isolated hypothyroxinemia % (n)	0	1.80% (13)	2.22% (15)	2.91% (9)	0.42
Subclinical hypothyroidism % (n)	7.47% (123)	1.60% (6) [*]	2.22% (15) [*]	2.27% (7) [*]	< 0.001

Abbreviations: BMI, body mass index; GW, gestational week; TSH, thyrotropin; FT4, free thyroxine;

SF, serum ferritin; sTfR, soluble transferrin receptor; TBI, total body iron; UIC, urinary iodine concentration;

Age, BMI, GW and FT4 were expressed as Mean ± SDs.

TSH, SF, sTfR, TBI, UIC were expressed as Median (interquartile range).

Enumeration data were presented as percentage (cases).

$P < 0.001$, a statistically significant difference between the four groups.

a, b, c, d : a statistically significant difference between the four groups using Kruskal-Wallis H test of ranked groups, $P < 0.05$.

*: compared with the non-pregnant women, $P < 0.01$.

Table 2. Comparison of serum FT₄ levels and rate of hypothyroxinemia between the pregnant women with or without ID in the different trimesters of pregnancy

Stage	Serum FT ₄		Rate of hypothyroxinemia	
	ID	Iron adequacy	ID	Iron adequacy
T1 N = 723	15.00±1.99** (n = 34)	16.35±1.95 (n = 689)	8.82%* (n = 34)	1.45% (n = 689)
T2 N = 645	11.30±3.02** (n = 42)	12.58±2.08 (n = 633)	11.90%** (n = 42)	2.37% (n = 633)
T3 N = 309	10.87±1.49 (n = 144)	11.12±1.66 (n = 165)	2.08% (n = 144)	3.64% (n = 165)

Abbreviations: FT₄, free thyroxine; ID, iron deficiency; T1, first trimester; T2, second trimester; T3, third trimester;

** $p < 0.01$, compared with iron adequacy.

* $p < 0.05$, compared with iron adequacy.

The trimester-specific hypothyroxinemia was defined as FT₄ levels below the 2.5th percentile of reference range with thyrotropin (TSH) levels in the normal reference range (P2.5th - P97.5th percentiles) or in the higher 97.5th percentile of reference range in each trimester of pregnancy.

ID was defined as serum ferritin (SF) less than 12 µg/L, or soluble transferrin receptor (sTfR) higher than 4.4 mg/L, or total body iron (TBI) less than 0 mg/kg.

Table 3. Multiple linear regression analysis of FT4 and related risk factors in different trimesters of pregnancy

		β	P
First trimester (n = 723)	Constant	18.93	< 0.001
	TBI	0.082	0.001
	BMI	-0.145	< 0.001
	Gestational week	-0.046	0.498
	Iodine	-0.001	0.011
	Age	0.013	0.555
Second trimester (n = 675)	Constant	20.17	< 0.001
	TBI	0.067	0.005
	Gestational week	-0.345	< 0.001
	Iodine	0.000	0.755
	Age	-0.060	0.003
Third trimester (n = 309)	Constant	11.64	< 0.001
	TBI	0.029	0.400
	Gestational week	-0.018	0.862
	Iodine	-0.002	0.015
	Age	0.006	0.855

Abbreviations: TBI, total body iron; BMI, body mass index;

Table 4. Evaluation of the risk factors for hypothyroxinemia in different trimesters of pregnancy using multiple logistic regression analysis

	First trimester		Second trimester		Third trimester	
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Maternal age	0.84 (0.71-0.99)	0.042	1.15 (1.03-1.29)	0.015	1.10 (0.91-1.34)	0.317
Gestational week	1.08 (0.53-2.23)	0.827	1.53 (1.19-1.96)	0.001	0.75 (0.33-1.67)	0.476
ID	14.86 (2.31-95.81)	0.005	3.36 (1.01-11.21)	0.048	0.59 (0.14-2.53)	0.484
Increased BMI	14.50 (2.65-79.32)	0.002				
Excessive iodine	9.77 (1.35-70.95)	0.024	0.95 (0.28-3.22)	0.94	11.05(0.5-250.06)	0.107

Abbreviations: CI, confidence interval; ID, iron deficiency; BMI, Body mass index;

ID was defined as serum ferritin (SF) < 12 µg/L, or soluble transferrin receptor (sTfR) > 4.4 mg/L, or total body iron (TBI) less than 0 mg/kg.

Increased BMI: BMI ≥ 25 kg/m², vs BMI < 25 kg/m².

Excessive iodine: urinary iodine > 500 µg/L, vs urinary iodine 150-250 µg/L.

First trimester: maternal age, gestational week, ID, BMI and urinary iodine were used as independent variables in the logistic regression model.

Second and third trimesters: maternal age, gestational week and ID were used as independent variables in the logistic regression model. BMI was not available for the second and third trimesters, and thus not included in Table 4.

The trimester-specific hypothyroxinemia was defined as free thyroxine (FT₄) levels below the 2.5th percentile of reference range with thyrotropin (TSH) levels in the normal reference range (P2.5th - P97.5th percentiles) or in the higher 97.5th percentile of reference range in each trimester of pregnancy.

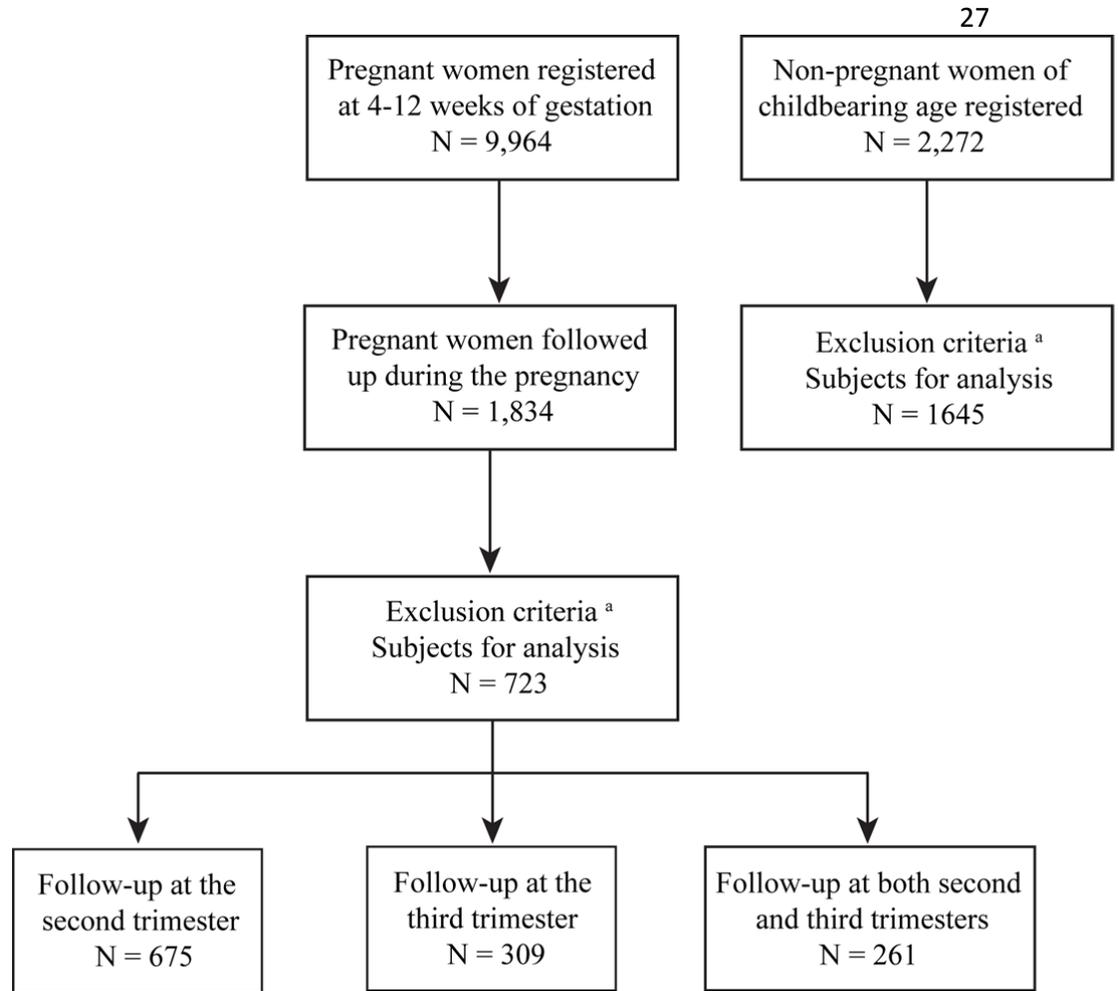


Figure 1. The flow diagram for the procedure of the subjects selected.

^a : Excluding the subjects with self-reported blood diseases, infection or fever history on collecting vein blood, positive thyroid autoantibodies, drugs treatment or supplementation containing iron or iodine, and L-T4 or anti-thyroid drugs treatment during both the registration and pregnancy.

Supplemental Table 1. Comparison of FT4 levels and rate of hypothyroxinemia in the second trimester between the pregnant women previously diagnosed with or without ID during the first trimester of pregnancy

	With ID	Without ID
Serum FT4	11.39±1.84** (n=32)	12.55±2.17 (n=643)
Rate of hypothyroxinemia	6.25% (2/32) (n=32)	2.80% (18/643) (n=643)

Abbreviations: FT4, free thyroxine; ID, iron deficiency;

** $p < 0.01$, compared with iron adequacy.

The trimester-specific hypothyroxinemia was defined as FT4 levels below the 2.5th percentile of reference range with serum thyrotropin (TSH) levels in the normal or higher than the 97.5th percentile of reference range in each trimester of pregnancy.

Supplemental Table 2. Comparison of FT4 levels and rate of hypothyroxinemia in the third trimester between pregnant women previously diagnosed with or without ID during the first trimester of pregnancy

	With ID	Without ID
Serum FT4	9.93±1.51* (n = 13)	10.96±1.49 (n = 294)
Rate of of hypothyroxinemia	6.67% (1/15) (n = 15)	2.72% (8/294) (n = 294)

Abbreviations: FT4, free thyroxine; ID, iron deficiency;

* $P = 0.016$, compared with iron adequacy.

The trimester-specific hypothyroxinemia was defined as FT4 levels below the 2.5th percentile of reference range with serum thyrotropin (TSH) levels in the normal or higher than the 97.5th percentile of reference range in each trimester of pregnancy.

Supplemental Table 3. Comparison of FT4 levels and rate of hypothyroxinemia in the third trimester between the pregnant women previously diagnosed with or without ID during the second trimester of pregnancy

	ID	Without ID
Serum FT4	10.74±1.51 (n = 18)	10.92±1.50 (n = 243)
Rate of of hypothyroxinemia	0 (0/18) (n = 18)	3.70% (9/243) (n = 243)

Abbreviations: FT4, free thyroxine; ID, iron deficiency;

The trimester-specific hypothyroxinemia was defined as FT4 levels below the 2.5th percentile of reference range with serum thyrotropin (TSH) levels in the normal or higher than the 97.5th percentile of reference range in each trimester of pregnancy.