Association between iron deficiency and prevalence of thyroid autoimmunity in pregnant and non-pregnant women of childbearing age: a cross-sectional study

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Abstract
Background: Thyroid autoimmunity (TAI) is prevalent among women of reproductive age and associated with adverse pregnancy outcomes. This study aimed to investigate the association between iron nutritional status and the prevalence of TAI in women during the first trimester of pregnancy and in non-pregnant women of childbearing age.

Methods: Cross-sectional analysis of 7463 pregnant women during the first trimester of pregnancy and 2185 non-pregnant women of childbearing age nested within the sub-clinical hypothyroid in early pregnancy study, a prospective collection of pregnant and non-pregnant women’s data, was conducted in Liaoning province of China between 2012 and 2015. Serum thyrotropin, free thyroxine, thyroid peroxidase antibodies (TPOAbs), thyroglobulin antibodies (TgAbs), serum ferritin, and urinary iodine were measured. Iron deficiency (ID) was defined as serum ferritin <15 μg/L and iron overload (IO) was defined as ferritin >150 μg/L. TPOAb-positive was defined as >34 U/mL and TgAb-positive was defined as >115 U/mL. Multilevel logistic regression was conducted to examine the association between TAI and different iron nutritional status after adjusting for potential confounders.

Results: The prevalence of isolated TPOAb-positive was markedly higher in women with ID than those without ID, in both pregnant and non-pregnant women (6.28% vs. 3.23%, χ² = 10.264, P = 0.002; 6.25% vs. 3.70%, χ² = 3.791, P = 0.044; respectively). After adjusting for confounders and the cluster effect of hospitals, ID remained associated with TPOAb-positive in pregnant and non-pregnant women (odds ratio [OR]: 3.591, 95% confidence interval [CI]: 1.241–3.919, P = 0.006; and OR: 1.822, 95% CI: 1.011–2.828, P = 0.046, respectively).

Conclusion: ID was associated with a higher prevalence of isolated TPOAbs-positive, but not with isolated TgAb-positive, in both pregnant women during the first trimester of pregnancy and non-pregnant women of childbearing age, while IO was not associated with either isolated TPOAb-positive or isolated TgAb-positive.

Clinical trial registration: ChiCTR-TRC-13003805, http://www.chictr.org.cn/index.aspx.
Keywords: Iron deficiency; Iron overload; Thyroid autoimmunity; Cross-sectional study; Pregnant; Childbearing age

Introduction
Thyroid dysfunction and thyroid autoimmunity (TAI) are prevalent among women of reproductive age and are associated with adverse pregnancy outcomes.[1] The importance of minerals, such as selenium and iron, to thyroid function has been extensively studied. Iron deficiency (ID) has multiple adverse effects on thyroid metabolism.[2-4] It decreases circulating thyroid hormone concentrations, likely through the impairment of the heme-dependent thyroid peroxidase (TPO) enzyme.[5] ID blunts the efficacy of iodine prophylaxis,[6] and iron repletion improves the efficacy of iodized salt in goitrous children with ID.[7] ID may influence iodine deficiency disorders through alterations of the central nervous system control of thyroid metabolism[4] or through modification of nuclear triiodothyronine (T3) binding.[8] Iron overload (IO) can induce the production of reactive oxygen species and the development of oxidative stress, which has been suggested to be able to induce inflammatory changes.[9]

However, the relationship between different status of iron and TAI has not been well investigated. This study was to...
address the association between ID or IO and the prevalence of TAI in pregnant women during the first trimester of pregnancy and non-pregnant women of childbearing age in an iodine-adequate region.

Methods

Ethical approval

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of China Medical University. Informed written consent was obtained from all individual participants prior to their enrollment in this study. This project has been registered in the World Health Organization’s International Clinical Trial Registry Platform (3 number: ChiCTR-TRC-13003805, http://www.chictr.org.cn/index.aspx).

Subjects

In this study, we reported on the data of a cross-sectional analysis of 7463 pregnant women and 2185 non-pregnant women, which was nested within the sub-clinical hypothyroid in early pregnancy (SHEP) study, a prospective collection of women’s obstetrical parameters and biological data. SHEP was conducted in Shenyang, Dalian, Dandong of Liaoning province, where the iodine status was adequate, as described by Li et al.[10] Nineteen grade-A tertiary hospitals located in the city center were involved in this study. Recruitment criteria included: residence in the local area for more than 10 years, age between 19 and 40 years, and having a singleton pregnancy at 4 to 12 weeks of gestation or planning to become pregnancy. Exclusion criteria included: multiple pregnancies, a history of thyroid disease or any other chronic diseases, taking oral contraceptive regimens or any medical regimen that may affect thyroid function, such as dopamine, or anti-epileptic drugs, as previously described by Li et al.[10]

A total of 9964 pregnant women in the first trimester of pregnancy and 2272 non-pregnant women of childbearing age participated in the study. All participants were asked to complete the questionnaires concerning personal information, such as the personal and family history of thyroid diseases, parity, smoking and drinking, chronic diseases, and multiple-micronutrient supplementation. Their fasting serum and urine samples were collected at the first visit. Serum thyrophin (TSH), free thyroxine (FT4), thyroid peroxidase antibodies (TPOAbs), thyroglobulin antibodies (TgAbs), and serum ferritin (SF) were measured.

Based on the questionnaires, two non-pregnant women and 60 pregnant women were excluded from the study due to having a history of hyperthyroidism or hypothyroidism. In addition, 29 non-pregnant women and 2435 pregnant women were excluded from the study due to taking supplementation containing iron at screening. Six pregnant women were excluded from the study due to having multiple pregnancies. Fifty-six non-pregnant women were excluded for privacy reasons. Finally, a total of 7463 pregnant women and 2185 non-pregnant women of childbearing age were included in the study [Figure 1].

Laboratory examinations

Samples of spot urine and fasting blood were obtained from each participant in the morning. All specimens were frozen at –20°C until shipment and assay in 1 week. Serum TSH, FT4, TPOAbs, TgAbs, and SF were measured by the electrochemiluminescence immunoassay with Cobas Eleys 601 (Roche Diagnostics, Basel, Switzerland). The intra-assay coefficients of variation (CVs) of serum TSH, FT4, TPOAbs, TgAbs, and SF were 1.57% to 4.12%, 2.24% to 6.33%, 2.42% to 5.63%, 1.30% to 4.90%, and 1.43% to 4.52%, respectively. The inter-assay CVs were 1.26% to 5.76%, 4.53% to 8.23%, 5.23% to 8.16%, 2.10% to 6.90%, and 3.52% to 7.91%, respectively. Urinary iodine (UI) was determined by the ammonium persulfate method based on the Sandell-Kolthoff reaction. The intra- and inter-assay CVs of UI were 3.00% to 4.00% and 4.00% to 6.00% at 66 g/L, respectively; and 2.00% to

Figure 1: The flow diagram of this study.
5.00% and 3.00% to 6.00% at 230 g/L, respectively. In the present study, the reference intervals of TSH were 0.14 to 4.87 mU/L and 0.69 to 5.64 mU/L for pregnant and non-pregnant women, respectively as previously described by Li et al.[10]. The manufacturer-specified population reference stages were 0 to 34 U/mL for TPOAbs and 0 to 115 U/mL for TgAbs. Women were divided into ID, IO, and control group. ID was defined as SF < 15 µg/L, and IO was defined as SF > 150 µg/L, and everyone in between served as control group.[11] Women with ID were further divided into two groups. We defined mild ID as 5 µg/L < SF < 15 µg/L, severe ID as SF ≤ 5 µg/L.

**Statistical analysis**

Statistical analyses were performed using IBM SPSS version 21.0 (IBM Corp., Armonk, NY, USA) and SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). The Kolmogorov-Smirnov method was used to test normality of the data distribution. The Kolmogorov-Smirnov method was used to test normality of the data distribution. The non-pregnant women of childbearing age were of median age 230 (16.34 ± 3.26 pmol/L) vs. 15.74 ± 3.26 pmol/L, t = 2.511, P = 0.012), compared to non-pregnant women. The prevalence of ID was lower in pregnant women than non-pregnant women (5.15% vs. 11.72%, χ² = 117.813, P < 0.001) [Table 1].

**Relationship between iron status and thyroid function in pregnant women during the first trimester and non-pregnant women of childbearing age**

Compared with the control group, the level of serum FT4 was lower in the ID group (pregnant women: 15.77 ± 1.99 pmol/L vs. 16.30 ± 3.13 pmol/L, t = 3.295, P = 0.001; non-pregnant women: 14.00 ± 2.66 pmol/L vs. 15.80 ± 3.26 pmol/L, t = 3.775, P < 0.001), but was higher in the IO group (pregnant women: 17.02 ± 3.17 pmol/L vs. 16.30 ± 3.13 pmol/L, t = 3.498, P < 0.001; non-pregnant women: 16.49 ± 3.09 pmol/L vs. 15.80 ± 3.26 pmol/L, t = 3.13, P = 0.03) in both pregnant and non-pregnant women [Table 2]. The median TSH levels of the IO group were similar to those of the control groups in both pregnant and non-pregnant women and were lower in the IO group (1.66 [1.00, 2.60] µg/L vs. 1.83 [1.14, 2.35] µg/L, Z = –3.345, P = 0.001) than that in the control group in pregnant women [Table 2].

**Table 1: Characteristics of pregnant women in the first trimester and non-pregnant women of childbearing age.**

| Characteristics | Pregnant women (N = 7463) | Non-pregnant women (N = 2185) | Statistics | P |
|-----------------|--------------------------|-------------------------------|------------|---|
| Age (years)     | 28.7 ± 3.8               | 28.0 ± 3.5                    | 1.304<sup>*</sup> | 0.195 |
| BMI (kg/m²)     | 21.9 ± 3.4               | 21.7 ± 3.8                    | –1.220<sup>†</sup> | 0.222 |
| Laboratory tests|                          |                               |            |    |
| TSH (mU/L)      | 1.82 (1.12, 2.74)         | 2.19 (1.54, 3.06)             | –13.721<sup>‡</sup> | <0.001 |
| FT4 (pmol/L)    | 16.34 ± 3.32             | 15.74 ± 3.26                  | 2.511<sup>†</sup> | 0.012 |
| TPOAb (U/mL)    | 7.28 (5.00, 11.43)        | 9.29 (6.18, 14.12)            | –14.648<sup>‡</sup> | <0.001 |
| TgAb (U/mL)     | 11.40 (10.00, 24.71)      | 11.69 (10.00, 25.34)          | –1.458<sup>†</sup> | 0.145 |
| SF (µg/L)       | 63.38 (39.16, 98.82)      | 49.00 (26.70, 78.78)          | 14.751<sup>‡</sup> | <0.001 |
| UI (µg/L)       | 152.33 (106.12, 212.69)   | 160.32 (98.64, 246.69)        | –0.891<sup>†</sup> | 0.373 |
| Prevalence ID   | 5.15 (384/7463)           | 11.72 (256/2185)              | 117.813<sup>‡</sup> | <0.001 |
| Isoalted TPOAb-positive | 3.40 (254/7463)   | 3.94 (862/2185)              | 1.409<sup>†</sup> | 0.235 |
| Isolated TgAb-positive | 6.91 (516/7463) | 7.00 (153/2185)              | 0.020<sup>†</sup> | 0.886 |

The data are expressed as mean ± standard deviation, median (Q1, Q3), or % (N%). † t test, ‡ Mann-Whitney U test, *Chi-square test. BMI: Body mass index; TSH: Thyrotropin; FT4: Free thyroxine; TPOAb: Thyroid peroxidase antibody; TgAb: Thyroglobulin antibody; SF: Serum ferritin; UI: Urinary iodine; ID: Iron deficiency.
Table 2: Thyroid function and prevalence of thyroid diseases in pregnant women and non-pregnant women with different iron levels.

| Items                        | Pregnant women |                  | Non-pregnant women |                  |
|------------------------------|----------------|------------------|--------------------|------------------|
|                              | Iron deficiency | Control          | Iron overload      |                  |
|                              | (n = 382)       | (n = 641)        | (n = 664)          |                  |
| Thyroid parameters           |                |                  |                    |                  |
| TSH (mIU/L)                  | 1.87 (1.17, 2.61) | 1.83 (1.14, 2.35) | 1.66 (1.00, 2.66) | 2.26 (1.64, 3.09) |
| FT4 (pmol/L)                 | 15.77 ± 1.99   | 16.30 ± 1.13     | 17.02 ± 3.17       | 14.00 ± 2.66     |
| TPOAbs (U/mL)                | 8.22 (5.00, 13.33) | 7.26 (5.00, 11.35) | 6.93 (5.00, 11.09) | 9.59 (6.40, 15.60) |
| TgAbs (U/mL)                 | 12.24 (10.00, 30.85) | 11.24 (10.00, 24.34) | 11.82 (10.00, 23.68) | 12.43 (10.00, 42.45) |
| UI (µg/L)                    | 157.31 (104.09, 204.33) | 152.58 (106.30, 214.20) | 145.31 (104.53, 204.33) | 154.38 (95.64, 261.79) |
| Prevalence                   |                |                  |                    |                  |
| Isolated TPOAbs-positive     | 6.28 (24)      | 3.23 (207)       | 3.46 (23)          | 6.25 (16)        |
| Isolated TgAbs-positive      | 7.59 (29)      | 6.90 (443)       | 6.63 (44)          | 5.08 (13)        |
| TPOAbs-positive and TgAbs-positive | 6.81 (26) | 5.50 (335) | 5.57 (37) | 9.77 (25) |
|                              |                |                  |                    |                  |
| The data are expressed as mean ± standard deviation, median (Q1, Q3), or % (n). *P < 0.01, compared with the pregnant women in control group. †P < 0.05, compared with the non-pregnant women in control group. TSH: Thyrotropin; FT4: Free thyroxine; TPOAbs: Thyroid peroxidase antibody; TgAbs: Thyroglobulin antibody; UI: Urinary iodine.

Relationship between iron status and TAI in pregnant women during the first trimester of pregnancy and non-pregnant women of childbearing age

The level of median TPOAbs was higher in ID group than that in the control group (8.22 vs. 5.00, 13.33) µg/L, Z = 2.690, P = 0.007 in the pregnant women. The prevalences of isolated TPOAbs-positive were higher in ID group than control groups in both pregnant (6.28% vs. 3.23%, χ² = 10.264, P = 0.002) and non-pregnant women (6.25% vs. 3.70%, χ² = 3.791, P = 0.044) [Table 2]. Furthermore, we divided ID group into mild ID group and severe ID group. We found that with the decrease of iron content, the prevalence of isolated TPOAbs-positive showed an increase trend in the pregnant women [Figure 2]. The prevalence of isolated TPOAbs-positive was 11.76% and 5.75% in severe and mild ID groups, respectively in the pregnant women. Both of these results were significantly higher than the control group (severe: 11.76% vs. 3.23%, χ² = 7.793, P = 0.024; mild: 5.75% vs. 3.23%, χ² = 6.470, P = 0.013). The prevalence of isolated TPOAbs-positive was 7.48% in mild ID group and was significantly higher than the control group (7.48% vs. 3.70%, χ² = 6.954, P = 0.016) in non-pregnant women [Figure 2].

Both the median level of TgAbs and the prevalence of isolated TgAbs-positive were similar between the ID group and the control group in both pregnant and non-pregnant women [Table 2]. We also stratified ID group into two groups, as described above, to investigate the prevalence of isolated TgAbs-positive in both pregnant and non-pregnant women. However, we did not observe a dose-dependent relationship between the prevalence of isolated TgAbs-positive and ID [Figure 2].

In the present study, neither the prevalence of TPOAbs-positive nor TgAbs-positive had statistically difference between the IO group and the control group in pregnant and non-pregnant women.

Multivariable and multilevel logistic regression analysis

After adjusting for confounding parameters in multivariable logistic regression, ID remained associated with isolated TPOAbs-positive in both pregnant (OR: 1.950, 95% CI: 1.266–3.003, P = 0.002) and non-pregnant women (OR: 1.878, 95% CI: 1.042–3.382, P = 0.036). However, ID was not associated with isolated TgAbs-positive in both pregnant and non-pregnant women [Tables 3 and 4]. To explore the cluster effect of 19 hospitals, we tested the random-effects parameters. The results suggested that there was a substantial cluster effect among 19 hospitals. Therefore, we further conducted multilevel regression analysis to adjust the cluster effect of hospitals. We found that multilevel regression analysis showed similar findings. ID remained associated with isolated TPOAbs-positive in both pregnant (OR: 2.111, 95% CI: 1.372–3.262, P = 0.001) and non-pregnant women (OR: 1.822, 95% CI: 1.011–3.282, P = 0.046). ID was not associated with isolated TgAbs-positive in both pregnant and non-pregnant women [Tables 3 and 4].

Discussion

ID is the most common nutritional disorder worldwide, and its prevention has been a public health goal. Studies in animals and human have shown that ID impairs thyroid metabolism by decreasing the activity of TPO and interfering with the synthesis of thyroid hormones, thereby leading to decreased plasma thyroxine (T4) and T3 concentration, as well as reduced peripheral conversion of T4 to T3.1,5,12,13 TAI is a multifactorial disease that has been associated in both spontaneous and assisted pregnancies with impaired outcomes, including miscarriage, preterm delivery, and lower birth weight.13,14 Previous studies showed that low selenium levels might increase the risk of developing TAI.13,16 However, the association between iron and TAI has not been clarified yet.

ID was previously reported to be associated with a higher prevalence of TPOAbs-positive in pregnant women during the first trimester of pregnancy.17,18 However, TgAbs were not measured and only pregnant women were analyzed in their studies. In the present study, we measured both TPOAbs and TgAbs in the pregnant women during the first trimester of pregnancy and non-pregnant women.
of childbearing age. We found an increased prevalence of isolated TPOAb-positive in both pregnant and non-pregnant women with ID, which was consistent with the studies of Veltri et al. [17] and Li et al. [18]. Moreover, we found that the more severe of ID, the higher of the prevalence of isolated TPOAb-positive in the pregnant women. However, we did not find ID was related to isolated TgAb-positive in both pregnant and non-pregnant women.

The mechanism behind ID was associated with isolated TPOAb-positive, but not TgAb-positive remains unclear. Iodine excess has been confirmed to be associated with the TgAb-positive, but not TPOAb-positive. [19-21] Previous animal studies reported that high dosage and prolonged iodine intake might lead to a rise in serum TgAbs and the lymphocytes infiltration. [22] The mechanism behind this phenomenon is explained by the fact that iodine plays a major role in the biological process of thyroglobulin (Tg).

![Figure 2: The prevalence of isolated TPOAb-positive and isolated TgAb-positive at different serum ferritin levels in pregnant women (n = 7463) and non-pregnant women (n = 2185). (A) The prevalence of isolated TPOAb-positive was higher in both the mild ID group (5.75%) and the severe ID group (11.76%) compared with the control group (3.23%) in pregnant women. (B) The prevalence of isolated TPOAb-positive was similar at different levels of serum ferritin in pregnant women. (C) The prevalence of isolated TPOAb-positive was higher in the mild ID group (7.48%) compared with the control group (3.70%) in non-pregnant women. (D) The prevalence of isolated TgAb-positive was similar at different levels of serum ferritin in non-pregnant women. *P < 0.050. Mild ID: 5 µg/L < SF < 15 µg/L; severe ID: SF ≤ 5 µg/L; control group: 15 µg/L < SF ≤ 150 µg/L; IO > 150 µg/L. ID: Iron deficiency; IO: Iron overload; TPOAb: Thyroid peroxidase antibody; TgAb: Thyroglobulin antibody.]

![Table 3: Multivariable logistic regression and multilevel regression analyses in pregnant women.]

| Items                | Isolated TPOAb-positive | Isolated TgAb-positive |
|----------------------|-------------------------|------------------------|
|                      | OR (95% CI)             | Multilevel OR (95% CI) | P     | OR (95% CI)             | Multilevel OR (95% CI) | P     |
| Age                  | 1.004 (0.968–1.042)     | 0.828                  | 0.196 | 1.003 (0.803–1.251)     | 0.981                  | 0.343 |
|                       | BMI*                   |                        |       |                       |                        |       |
| ≥25.0 kg/m²           | 1.055 (0.708–1.573)     | 0.792                  | 0.304 | 0.170 (0.865–1.583)     | 0.308                  | 0.689 |
| < 18.5 kg/m²          | 1.402 (0.871–2.258)     | 0.165                  | 0.853 | 1.072 (0.729–1.578)     | 0.723                  | 0.624 |
| Head of weeks         | 1.001 (0.983–1.016)     | 0.947                  | 0.279 | 1.003 (0.996–1.010)     | 0.452                  | 0.239 |
| Iron deficiencies     | 1.950 (1.266–3.003)     | 0.002                  | 0.066 | 1.197 (0.795–1.802)     | 0.389                  | 0.633 |
| < 150 µg/L            | 1.115 (0.442–2.813)     | 0.818                  | 0.742 | 0.825 (0.465–1.466)     | 0.513                  | 0.144 |
| 250–500 µg/L          | 1.822 (0.735–4.517)     | 0.195                  | 0.742 | 1.147 (0.653–2.013)     | 0.633                  | 0.961 |
| > 500 µg/L            | 1.723 (0.665–4.460)     | 0.262                  | 0.715 | 0.949 (0.515–1.748)     | 0.867                  | 0.823 |

*Compared with 18.5 kg/m² ≤ BMI < 25.0 kg/m². †Compared with 150 µg/L ≤ urinary iodine < 250 µg/L. TPOAb: Thyroid peroxidase antibody; TgAb: Thyroglobulin antibody; OR: Odds ratio; CI: Confidence interval; BMI: Body mass index.
Table 4: Multivariable logistic regression and multilevel regression analyses in non-pregnant women.

| Items                | Isolated-TPOAb-positive OR (95% CI) | Multilevel OR (95% CI) | P     | Isolated-TgAb-positive OR (95% CI) | Multilevel OR (95% CI) | P     |
|----------------------|------------------------------------|------------------------|-------|-----------------------------------|------------------------|-------|
| Age                  | 1.554 (0.921–2.622)                | 0.099                  | 1.023 (0.996–1.050) | 0.094                          | 1.059 (0.694–1.617)    | 0.790 | 1.014 (0.994–1.035) | 0.167 |
| BMI                  |                                    |                        |       |                                   |                        |       |                     |       |
| ≥25.0 kg/m²          | 1.638 (0.738–3.637)                | 0.225                  | 1.106 (0.610–2.006) | 0.740                          | 1.302 (0.858–2.627)    | 0.154 | 0.693 (0.421–1.141) | 0.150 |
| <18.5 kg/m²          | 1.882 (0.749–4.730)                | 0.179                  | 0.655 (0.294–1.457) | 0.300                          | 0.999 (0.486–2.052)    | 0.998 | 0.818 (0.487–1.374) | 0.447 |
| Iron deficiency      | 1.878 (1.042–3.382)                | 0.036                  | 1.822 (1.011–3.282) | 0.046                          | 0.675 (0.363–1.254)    | 0.214 | 0.753 (0.416–1.363) | 0.349 |
| Urinary iodine       |                                    |                        |       |                                   |                        |       |                     |       |
| <150 μg/L            | 1.216 (0.354–4.181)                | 0.756                  | 1.181 (0.683–2.043) | 0.551                          | 0.916 (0.443–1.942)    | 0.818 | 0.946 (0.642–1.394) | 0.778 |
| 250–500 μg/L         | 1.496 (0.451–4.948)                | 0.511                  | 1.409 (0.749–2.651) | 0.287                          | 0.822 (0.394–1.714)    | 0.601 | 0.863 (0.527–1.413) | 0.558 |
| >500 μg/L            | 1.613 (0.464–5.600)                | 0.453                  | 0.664 (0.188–2.351) | 0.526                          | 0.673 (0.299–1.512)    | 0.337 | 1.327 (0.671–2.621) | 0.416 |

*Compared with 18.5 kg/m² ≤ BMI < 25.0 kg/m², ‡Compared with 150 μg/L ≤ urinary iodine < 250 μg/L. TPOAb: Thyroid peroxidase antibody; TgAb: Thyroglobulin antibody; OR: Odds ratio; CI: Confidence interval; BMI: Body mass index.

Tg combined with high iodine can enhance its immunogenicity by generating novel epitopes or unmasking of a cryptic epitope on Tg.[23-25]

TPO, that has iron at its active center, is required for thyroid hormone synthesis.[5,26] Previous studies found that ID could impair the iron-dependent TPO activity, resulting in the decrease of serum thyroxine levels.[27] Unfortunately, the level of TPOAbs was not measured. Enlightened by the association between iodine excess and TgAb-positive, we speculated that TPO undergoes post-translational modification as a consequence of ID, with the exposure of previously hidden epitopes or generating novel epitopes, which enhances the immunogenicity of TPO. However, this speculation could not be proven by the current study. Another explanation for the association between ID and TPOAb-positive provided by Veltri et al[17] was that the lower TPO activity caused the increased prevalence of TAI in order to preserve its function.

Previous studies indicated IO could modulate and exaggerate the autoimmune process.[28,29] IO could induce the production of reactive oxygen species, the development of oxidative stress and lipid peroxidation, which would lead to demyelination in some autoimmune diseases, such as autoimmune encephalomyelitis and multiple sclerosis.[28] IO was also reported to be associated with the high prevalence of type 2 diabetes and gestational diabetes.[31,32] However, the association between IO and TAI has not yet been studied. In the present study, we found that neither isolated TPOAb-positive nor isolated TgAb-positive was associated with IO in both pregnant women and non-pregnant women of childbearing age.

There were several advantages in the current study. First, because iodine malnutrition can influence TAI, we performed the study in an iodine-adequate area. Furthermore, we excluded the factors that might interfere with the status of iron, such as anaemia and chronic inflammation. In this way, we better focused our analysis on the relationship between iron and TAI. We additionally used multilevel regression model to control for the cluster effect among different hospitals. Second, our study measured two antibodies representing TAI, TPOAbs, and TgAbs. We eliminated the confounding effect of these two antibodies on each other, thus clarifying the relationship between ID and isolated TPOAbs or isolated TgAbs. Third, our study analyzed the relationship between IO and TAI. To our knowledge, this was the large-scale epidemiological study to investigate the relationship between IO and TAI.

There were several limitations in the present study. First, this study did not measure soluble transferrin receptor (sTfR), so we could not calculate total body iron using SF and sTIR, which could correct the potential dilution effects secondarily to the expansion of blood volume. Second, this study was confined to iodine-adequate areas, and might not be generalized for the women in iodine-deficient or iodine-excessive areas. Third, the present study was a cross-sectional investigation, and we could not distinguish the causation between ID and isolated TPOAb-positive. Prospective randomized controlled trials are needed to clarify this causation.

In conclusion, this study showed that ID was associated with a higher prevalence of isolated TPOAb-positive, but not with isolated TgAb-positive, in both pregnant women during the first trimester of pregnancy and non-pregnant women of childbearing age, while IO was not associated with either TPOAb-positive or TgAs-positive. We speculated that ID may be a pathogenic factor for isolated TPOAb-positive in pregnant women and non-pregnant women of childbearing age. However, further prospective studies are needed to verify this.

**Conflicts of interest**

None.

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