Prevalence of thyroid autoimmunity and dysfunction in women with iron deficiency during early pregnancy: is it altered?

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Abstract

Objective: Thyroid disorders and iron deficiency (ID) are associated with obstetrical and fetal complications. Iron is essential for the normal functioning of thyroid peroxidase (TPO-abs) and ID is frequent during pregnancy. The aim of this study was to compare the prevalence of thyroid autoimmunity (TAI) and dysfunction during the first trimester of pregnancy in women with and without ID.

Design: Cross-sectional data analysis of 1900 pregnant women nested within an ongoing prospective collection of pregnant women’s data.

Method: The study was performed in a single, tertiary referral center. During the first antenatal visit, ferritin, TPO-abs, thyroid-stimulating hormone (TSH) and free T4 (FT4) were measured and age and BMI were recorded. ID was defined as ferritin <15 µg/L, TAI when TPO-abs was >60 kIU/L, and subclinical hypothyroidism (SCH) when TSH was >2.5 mIU/L.

Results: ID was present in 35% of women. Age and BMI were comparable between both groups. In the ID group, the prevalence of TAI and SCH was significantly higher, compared with that in the non-ID group (10% vs 6% and 20% vs 16%; P = 0.011 and 0.049 respectively). Ferritin was inversely correlated with serum TSH (ρ = −0.076; P = 0.001) and positive with FT4 levels (ρ = 0.112; P < 0.001). In the logistic regression model, ID remained associated with TAI after correction for confounding factors (P = 0.017). The association with SCH was absent after correction for the confounders in the logistic regression model (P = 0.082), but remained present in the linear regression model (P = 0.035).

Conclusions: ID was frequent during the first trimester of pregnancy and was associated with a higher prevalence of TAI, higher serum TSH, and lower FT4 levels.

Introduction

The importance of trace elements and minerals (copper, zinc, selenium and iron) for the normal functioning of the thyroid has extensively been studied and is well recognized. Iodine is essential for the synthesis of thyroid hormones and selenium is necessary for the deiodinase enzymes and to protect the thyroid against oxidative stress (1, 2). Iron also plays an important role in the normal functioning of thyroid peroxidase (TPO), a heme-dependent protein and it also facilitates the actions of iodine in the thyroid (3, 4). Iron deficiency (ID) remains a worldwide problem, affecting about 20% of the world’s population. ID arises when physiological requirements cannot be met by iron absorption from the diet such as it is the case during pregnancy, when iron needs are
Iron deficiency and thyroid disorders during pregnancy

F Veltri, S Decaillet and others

Tripled because of expansion of maternal red cell mass and growth of the fetus and placenta. In industrialized countries, the prevalence of ID during pregnancy ranges from 24 to 44% (5, 6). ID can be diagnosed by low serum ferritin levels, in the absence of inflammation, and it is the first parameter that changes when iron stores decrease, independently of recent iron intake. In women of reproductive age, a level <15 μg/L has a specificity of 98% and a sensitivity of 75% for ID (7).

The use of ferritin as a marker of peripheral thyroid hormone (in) sensitivity is well known and is used as a diagnostic tool in the differential diagnosis between thyrotropinoma and the thyroid hormone resistance syndrome (8). Data on the association between ferritin and thyroid disorders as outcome are limited to a few well-designed studies, of which one showed that ID was doubling the risk of developing hypothyroidism (9, 10). None of these studies investigated the impact of ID on thyroid autoimmunity (TAI). The association of ID and thyroid disorders during pregnancy is limited to one study measuring ID during the second and third trimester of pregnancy and a recent publication, linking ID with isolated hypothyroxinemia (11, 12). In the first study, TAI was not taken into account and in the second, the presence of TAI was an exclusion criterion.

The association between TAI/subclinical hypothyroidism (SCH) and increased fetal/obstetrical complications, the absence of studies investigating the prevalence of TAI in pregnant women with ID and finally the important role of iron for TPO functioning, were the main reasons to perform this study.

The aims of this study were to investigate the prevalence of ID during the first trimester of pregnancy and to investigate whether ID was an associated variable for the development of TAI and/or thyroid dysfunction. These observations were furthermore corrected for other confounders such as women’s BMI and age in two regression models.

Patients and methods

Overall study design

The obstetrical clinic of the CHU St-Pierre is a downtown university (tertiary referral) maternity in Brussels (Belgium), with about 3200 deliveries per year. In this study, we report on the data of a cross-sectional analysis of 1900 pregnant women (period 2013–2014), which was nested within the ongoing prospective collection of women’s obstetrical parameters and biological data. This study was approved by the institutional review board (AK/15-11-114/4568). In our center, a complete biological analysis is systematically prescribed at the first prenatal consultation (before 12th week of amenorrhea), including thyroid-stimulating hormone (TSH), free T4 (FT4), TPO- abs and iron status by the means of serum ferritin.

The diagnosis of an underlying thyroid disease was further evaluated based on the personal history of goiter, thyroid diseases, and/or prior use of thyroid medication and hereby 86 women were excluded from the study due to LT4 treatment. Women treated with iron supplements at the first antenatal visit were also excluded from the study (n=4). Files of pregnant women working in our institution were not included in the study for privacy reasons (n=114). Finally, 1900 files of pregnant women over the period 2013–2014 were included in the study (flowchart in Fig. 1). All over, there were less than 1% of the women smoking before pregnancy, and therefore, this variable was not taken into account.

The iron status was expressed in two groups of women (ID and non-ID); all other parameters were expressed as continuous values and categorical data (besides FT4).

Figure 1
Flowchart illustrating the selection of the finally included women in the study.
Iron deficiency and thyroid disorders during pregnancy

F Veltri, S Decaillet and others

ID was defined as a ferritin level <15 µg/L, TAI was present when TPO-abs were >60 IU/L, SCH when serum TSH levels were >2.5 mIU/L, obesity when BMI was ≥30 kg/m² and older women were defined when their age was ≥30 years. (Subclinical) hyperthyroidism was defined as a TSH level <0.10 mIU/L. The impact of the other variables, age, and BMI on TAI and SCH/TSH as outcomes was tested in a linear and logistic regression model.

Serum assay

All provisions were implemented by the laboratory of hormonology of our institution. Serum TSH, FT₄, TPO-abs and ferritin levels were measured using the Chemiluminescence Centaur XP Siemens immunoanalyzer. The reference values were 0.3–4.0 mIU/L, 0.8–2.0 ng/dL, <60 kIU/L and 15–300 µg/L for TSH, FT₄, TPO-abs and ferritin respectively. The total imprecision coefficient of variations (CVs) were 6.9, 4.2, 7.6 and 3.7% for TSH, FT₄, TPO-abs and ferritin respectively.

For conversion of FT₄, 1 ng/dL = 12.9 pmol/L.

Statistical analysis

Data were stored in a Microsoft Excel database and statistical analyses were performed using Stata 11.2 software (Lakeway Drive, TX, USA). Continuous data were all given as median (min–max) values, since they were not normally distributed. Categorical data were presented as number of cases (percentages). Differences between groups (ID, non-ID) were analyzed by Fisher’s exact tests for categorical data and by the Mann–Whitney U test for continuous data. Correlations between continuous variables were quantified using Spearman’s ρ correlation coefficient.

The impact of independent variables (ID, age, and BMI) on the dependent outcome measures (SCH and TAI) were explored by fitting logistic regression models. Similarly, potential confounding effects of age and BMI were explored by fitting multivariable regression models on log-transformed TSH values. All statistical tests were considered to be significant at P<0.05.

Results

Serum TSH levels and ferritin were inversely correlated (Spearman’s ρ = -0.076; P = 0.001) and a positive correlation was obtained between FT₄ and ferritin (Spearman’s ρ = 0.112; P < 0.001).

Figure 2 shows the graphical result of the univariable analysis, between log TSH as dependent and ferritin as independent variable.

Figure 3 shows the graphical result of the univariable analysis, between log FT₄ as dependent and ferritin as independent variable.

In the whole study group, suppressed TSH levels were present in 58 women (3.1%) and the number of cases were comparable between the ID group (n = 21, 3.1%) and the non-ID group (n = 37, 3.0%).

Table 1 shows ferritin levels, thyroid parameters, and demographic characteristics in all patients and stratified according to their iron status.

Serum ferritin levels (median (min–max)) were, by definition, significantly lower in the ID group as compared

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**Figure 2**
Illustration of the univariable analysis, with log TSH as dependent and ferritin as independent variable.

**Figure 3**
Illustration of the univariable analysis, with log FT₄ as dependent and ferritin as independent variable.
Iron deficiency and thyroid disorders during pregnancy

F Veltri, S Decaillet and others

Clinical Study

With the non-ID group (10 (1–14) vs 31 (15–335) µg/L; P<0.001). Serum TSH levels were significantly higher in the ID group as compared with the non-ID group (1.5 (0.0–9.6) vs 1.3 (0.0–30.5) mIU/L; P=0.015) and FT₄ levels were lower in the ID group as compared with the non-ID group (1.0 (0.7–2.2) vs 1.1 (0.6–3.1) ng/dL; P<0.001). Serum TPO-abs levels were comparable between both groups and this was also the case for the ages and BMI levels.

Table 1 shows the prevalence of SCH, TAI, women ≥30 years, and obesity in all patients and according to their iron status.

Table 2 shows the prevalence of thyroid autoimmunity, subclinical hypothyroidism and obesity in all patients and according to the iron status.

Table 3 shows the results of the univariable and multivariable logistic and linear regressions according to the different outcomes.

The prevalence of TAI and SCH was significantly higher in the group with ID as compared with that in the non-ID group (10% vs 6% and 20% vs 16%, P=0.011 and P=0.049 respectively). The prevalence of women ≥30 years and obese women (BMI ≥30 kg/m²) was comparable between both study groups.

Table 3 shows the results of the univariable and multivariable logistic and linear regressions according to the different outcomes.
Iron deficiency and thyroid disorders during pregnancy

Thyroid autoimmunity as categorical outcome

Univariable analysis
ID was associated with an increased risk for the TAI (odds ratio (OR) 1.57 (95% CI: 1.11–2.21); \( P = 0.009 \)).

SCH was also significantly associated (OR 2.31 (95% CI: 1.59–3.37); \( P < 0.001 \)). No association with age \( \geq 30 \) years (OR 1.39 (95% CI: 0.98–1.96); \( P = 0.060 \)) or BMI (OR 0.77 (95% CI: 0.48–1.25); \( P = 0.305 \)) was obtained.

Multivariable logistic regression analysis
After adjustment for confounding parameters in the final step forward logistic regression, ID remained associated with TAI (OR 1.52 (95% CI: 1.07–2.15); \( P = 0.017 \)), SCH (OR 2.32 (95% CI: 1.58–3.39); \( P < 0.001 \)) was also the independent variable associated with TAI.

When in the logistic multivariable analysis, age was added as a continuous variable, then it was associated with TAI (OR 1.05 (95% CI: 1.02–1.08); \( P < 0.001 \)). The associations with ID and SCH remained significant (data not shown).

Thyroid function (log TSH) as continuous outcome and subclinical thyroid dysfunction as categorical outcome

As categorical outcome (SCH): univariable analysis
ID was associated with a higher risk for SCH (OR 1.28 (95% CI: 1.00–1.64); \( P = 0.042 \)). Also age \( \geq 30 \) years (OR 0.75 (95% CI: 0.59–0.96; \( P = 0.023 \)) and TAI (OR 2.31 (95% CI: 1.59–3.37); \( P < 0.001 \)) were independently associated with a higher risk for SCH. No association with BMI was obtained (OR 0.91 (95% CI: 0.66–1.25; \( P = 0.595 \)).

Multivariable logistic regression analysis
After adjustment for confounding variables in the final step forward logistic regression, ID was no longer associated with a higher risk for SCH (OR 1.24 (95% CI: 0.97–1.59); \( P = 0.082 \)). Age \( \geq 30 \) years was associated with a lower risk for the presence of SCH (OR 0.73 (95% CI: 0.58–0.94; \( P = 0.014 \)) and especially the presence of TAI (OR 2.32 (95% CI: 1.59–3.40); \( P < 0.001 \)) was an independent variable associated with a higher risk for SCH.

When in the logistic multivariable analysis, age was added as a continuous variable, the results did not change the significant associations as compared with that in case age was used as a categorical independent variable (\( \geq 30 \) years; data not shown).

Discussion
The prevalence of ID in our cohort of pregnant women was high, indicating that even in 2016 in a metropolitan area, it remains an important problem. The prevalence we observed was in line with that in most other studies, although in a recent Chinese study, a lower prevalence of ID (<10%) was noticed. This discrepancy may be explained by different socioeconomical states (most of our patients are non-employed), by the fact that we excluded patients taking iron supplements at the time of ferritin measurement and finally because we measured ferritin levels later in pregnancy as compared with the Chinese study, in which it was measured before the 12th week of pregnancy (12).

Most studies did not take women’s BMI into account; a variable known to be inversely related with ferritin levels and thus increasing the prevalence of ID (13). In our study, however, no correlation between BMI and ferritin levels was obtained and the prevalence of obesity was comparable between women with and without ID. Our observations were furthermore comparable to those of a recent study in pregnant women in the UK (14).
We are not aware of another study, showing an increased prevalence of TAI in women with ID, an association that remained significant after correction for confounding factors. Previous studies did not take TAI into account, or used it as an exclusion criterion to investigate the relationship between ferritin and thyroid function (11, 12). The importance of our results lays in the fact that the presence of TAI has been associated in both spontaneous and assisted pregnancies with impaired outcomes, including (recurrent) miscarriage, preterm delivery, low birth weight and postpartum thyroiditis (15, 16).

The pathophysiological mechanisms for our observations remain largely speculative. One seemingly obvious explanation could be the association between (low) iron levels and (lower) TPO activity, as it has been described earlier in an animal model (3). It then could be speculated that the lower TPO activity causes the increased prevalence of TAI in order to try to preserve its function, but that cannot be proven with the current study design. TPO is a heme-dependent protein and when its activity is lower due to ID, the iodine incorporation into thyroglobulin (Tg) and the coupling of iodotyrosines to form thyroid hormones is impaired, leading to lower FT$_4$ production and higher serum TSH levels (17, 18, 19). In our analysis, we also showed that SCH is an independent and significant risk factor for the development of TAI. These higher levels of TSH might increase the presentation of follicular antigens and therefore also the increased TPO-ab levels. Whether these antigens are then only TPO, Tg, or both is also a question, we cannot answer because Tg and Tg-abs were not measured. Indeed, in a study by Unuane et al., it has been shown that in the case of TAI (when using sensitive assays), the prevalence of Tg-abs is even higher in women of reproductive ages as compared with that of TPO-abs (20).

In women older than 30 years, there was a trend to observe an increased risk for the development of TAI, in line with the known higher prevalence of TAI with increasing age (21). In our study, the median ages between women with and without ID were comparable and the oldest pregnant woman was 47-year old.

An important observation was that after correction for TSH and age, ID remained associated with an increased prevalence of TAI and therefore, other mechanisms must have been involved explaining this association. ID also lowers the activity of other heme-containing enzymes, such as cytochrome oxidase and myeloperoxidase (MPO) (3), and therefore one might speculate that women with ID develop antibodies against MPO more easily, leading to a cross-reaction with TPO-abs and subsequently explaining the higher prevalence of TAI (22). In a more recent paper, the cross-reactivity hypothesis could however not be confirmed (23). Data on the prevalence of TAI in pregnant women after iodine fortification (like the fortification program in Denmark) taught us that the prevalence of TAI increased 10 years later (24). In Belgium, however, attempts to increase the daily iodine intake are not opposed by the authorities and are still voluntary. Therefore, that hypothesis cannot be taken into account to explain the association of ID-TAI (25).

Finally, the presence of TAI goes far beyond the association with iron levels and that it has a complex immunopathogenesis, in which familial (genetic) and environmental factors play an important role (26).

The ID could also have been a consequence of the autoimmune thyroiditis. In a study a few years ago and recently confirmed by another group, concomitant autoimmune gastritis with TAI was present in 33% of patients (27, 28). Furthermore, it was shown in another study of patients with TAI that microcytic anemia was present in almost 20% of them, due to concomitant autoimmune gastritis and low ferritin levels (29). We did not measure other antibodies or included hemoglobin levels in our cohort of patients and can thus not draw conclusions on this issue. However, it is noteworthy that in the study by Zimmerman et al., anemia secondary to hypothyroidism was present in only 6% of their patients (11).

The other main observations in our cohort of women with ID were the increased serum TSH levels/ SCH prevalence and lower FT$_4$ levels. This association remained significant after correction for confounders such as age and BMI and even for TPO-abs. Our findings were concordant with those in literature (9, 10, 11). Potential mechanisms have already been discussed above in relation to a lower TPO activity. Furthermore, it has been shown that in case of ID, the control of the CNS can be altered, the binding of T$_3$ to hepatic nuclear receptors changed, oxygen transport altered, and finally the conversion from T$_4$ to T$_3$ can be impaired (3, 17, 18, 19).

In contrast with our findings, in a recent Chinese study no negative correlation between ID and serum TSH (12) was observed. According to the authors, this was due to the iodine sufficiency in the investigated region as compared with a mild iodine deficiency in the study by Zimmerman et al. (11). Also in our study, women had probably a mild iodine deficiency, as it was recorded in a survey performed in different obstetrical centers in the Brussels metropolitan area (including our center) (30).
Furthermore, the samples in the Chinese study were collected very early during pregnancy, when human chorionic gonadotropin (hCG) levels were the highest and it was possible to dampen the serum TSH levels (31).

A recent study showed that women with both ID and anemia were at higher risk to develop increased serum TSH levels as compared with ID alone (10). In the study by Zimmerman et al., the prevalence of anemia in case of ID was low and it did not influence the prevalence of SCH (11). We did not include hemoglobin levels in our cohort of pregnant women.

Another hormone that plays an important role in the iron metabolism is hepcidin. This has recently been studied in relation to thyroid function (although outside pregnancy) and the authors found a positive correlation between both parameters (32). This might imply that SCH leads to higher levels of hepcidin, the latter then blocking iron absorption. Low iron, in case of pregnancy, probably also leads to changes in hepcidin levels, a hormone and thus by definition acting on different target cells in the body; but to our best knowledge, an impact of hepcidin on the thyroid has not yet been described. In clinical practice, studies investigating the relationship between hepcidin and thyroid function are progressing slowly, in part due to the imperfect and often expensive assays for its measurement.

The significant association between ID and SCH we obtained in the univariable analysis could not be confirmed in the logistic regression model, due to the important impact of TAI on thyroid function. This was an expected finding, since TAI is causing SCH in 90% of patients in iodine sufficient areas (33). Age was also an independent variable, negatively associated with the presence of SCH. The reason for this rather unexpected finding is probably due to the inclusion of patients with a suppressed serum TSH in the group of pregnant women without SCH. In a recent paper, specifically investigating the prevalence of thyroid dysfunction in relation to women older than 30 years, no effect was noticed (34). Another reason why the results of the univariable analysis could not be confirmed might have been due to the 2.5 mIU/L cut-off level we used to define SCH in the categorical model. We did not dispose of institutional trimester specific cut-off levels for TSH during pregnancy and according to recommendations of the Endocrine Society on thyroid screening during pregnancy; we decided to use the 2.5 mIU/L TSH cut-off level (35).

Since TPO is a heme-dependent enzyme, the fact that iron supplements lead to an enhanced thyroid function and finally knowing that iodine supplements are more efficient when combined with iron, we believe that the relationship between iron and thyroid function exceeds that of an simple association and might be causal (4, 36). In daily practice, pregnant women often receive multivitamins of which some contain iron (18 mg) and iodine (150 µg). In this regard, it also deserves attention that preparations containing iron should be separated from LT₄ intake by at least 4 h in order to avoid LT₄ malabsorption (37). To date, no studies in pregnant women have been published providing evidence that multivitamin complexes ameliorate thyroid function. ID as such is a known risk factor to develop obstetrical complication and, according to our current findings, this harmful impact might thus in part have been mediated through the thyroid pathway (38). It has indeed been shown that even a slight increased serum TSH within the normal range is associated with an increased first trimester miscarriage rate (39). In order to answer the question whether ID, TAI, SCH or a combination hampers a normal pregnancy evolution, prospective randomized trials are largely needed.

Concerning the screening for thyroid disorders in pregnant women, it has recently been shown that in case of target screening, 33% of women with positive TPO-abs would have been missed (40). In the ongoing debate between systematically versus targeted high-risk case screening, and when opting for the latter, based on our study findings, we believe that ID should be added as an additional risk factor in order to detect more women with TAI and thyroid dysfunction.

Our study is hampered by several limitations: we did not measure hemoglobin, total iron binding capacity (TIBC), the iron saturation index, Tg-abs, antiparietal antibodies, the familial occurrence of TAI was not recorded and finally, thyroid function tests were not corrected for hCG levels at the time of the blood samples. Our work does not provide clinical variables like parity or previous miscarriages, which would have helped to understand the high prevalence of ID. Furthermore, it should be mentioned that our linear regression model only explains <1% of the variability in log TSH, relativizing our findings. However, does the impact of ID on the prevalence of TAI seem to be more important and since TAI increases the risk to develop thyroid dysfunction during pregnancy and the rate of pregnancy complications, the observations remain important.

It is obvious that further prospective studies are needed in order to investigate whether our data can be confirmed and to try to explain the association between ID, TAI, and thyroid dysfunction in more detail and especially in relation to the pregnancy outcome.
Conclusion
ID is frequent during the first trimester of pregnancy and is associated with a higher prevalence of TAI, increased TSH, and lower FT4 levels, independent of confounding factors. ID should be added to the risk factors associated with the development of TAI and dysfunction during pregnancy.

Declaration of interest
K P received fees for lectures he gave at Merck symposia in 2011 and 2014. All other authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Iron deficiency and thyroid disorders during pregnancy

F Veltri, S Decaillet and others


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