Iron nutritional status is associated with thyroid hormone levels in the second trimester of pregnancy: a case-control study

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Abstract

Objective: To analyze the correlation between iron status and thyroid hormone in pregnant women during their second trimester of pregnancy. Design: A hospital-based case-control study. Population: A total of 550 pregnant women were recruited from Pingguo Maternity and Child Health Hospital. Method: Data was grouped by SF concentration, and was performed by correlation and regression analysis. Main outcome measure: Iron nutritional status and thyroid hormones. Results: A significant difference was found in thyroid hormone level, including FT3, FT4, and TSH (all $P < 0.01$) between two groups (Mann-Whitney U test), and the levels of FT3 and FT4 were significantly lower in the iron deficiency group. Spearman's correlation analysis showed that both of SF and Hb were correlated with FT3, FT4, and TSH (all $P < 0.01$). Canonical correlation was used to analyze the interrelationships between iron status and thyroid hormones, showing SF was positively associated with the FT3 level. The elastic network regression indicated that the coefficient of Hb was greater than that of SF. Conclusion: Our study indicated that iron nutritional status is associated with thyroid hormone levels during the second trimester of pregnancy, suggesting that ID may be an important risk factor in thyroid hormone levels. Involving two common endocrine diseases in pregnancy, the relationship between ID and thyroid hormone deserves further investigation. Tweetable abstract: Iron nutritional status is associated with thyroid hormone levels during the second trimester of pregnancy. Key-words: Iron status, thyroid hormones, the second trimester of pregnancy, canonical correlation, elastic network regression

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Short title: The relationship of thyroid and iron status in pregnancy

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Abstract

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Design: A hospital-based case-control study.

Population: A total of 550 pregnant women were recruited from Pingguo Maternity and Child Health Hospital.

Method: Data was grouped by SF concentration, and was performed by correlation and regression analysis.

Main outcome measure: Iron nutritional status and thyroid hormones.

Results: A significant difference was found in thyroid hormone level, including FT3, FT4, and TSH (all $P < 0.01$) between two groups (Mann-Whitney U test), and the levels of FT3 and FT4 were significantly lower in the iron deficiency group. Spearman's correlation analysis showed that both of SF and Hb were correlated with FT3, FT4, and TSH (all $P < 0.01$). Canonical correlation was used to analyze the interrelationships between iron status and thyroid hormones, showing SF was positively associated with the FT3 level. The elastic network regression indicated that the coefficient of Hb was greater than that of SF.

Conclusion: Our study indicated that iron nutritional status is associated with thyroid hormone levels during the second trimester of pregnancy, suggesting that ID may be an important risk factor in thyroid hormone levels. Involving two common endocrine diseases in pregnancy, the relationship between ID and thyroid hormone deserves further investigation.

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Introduction

Iron deficiency (ID) is one of the most common nutritional deficiencies, especially among pregnant women and children[1]. Pregnant women are likely to develop ID and ID anemia[2]. This is due to increased iron requirement following the enlargement of blood volume, which is increased during the pregnancy to maintain physiological metabolism of maternal tissues and to meet the needs of the fetus[3]. Iron nutrition has a profound influence on brain development and neurotransmitter formation during fetal development. The most striking effect of ID is the impairment of intellectual function[4]. ID can disrupt the brain development and lead to abnormal hippocampal structure and function; it also changes brain energy metabolism and neuronal signaling[4-6]. Therefore, early detection of ID and iron supplementation therapy are particularly important. Given that ID often leads to pregnant complications and affects the development of offspring, iron nutrition and anemia status in pregnant women have long been the focus of research.

Iron is an essential trace element in human body and plays an important role in the function of all cells, particularly in oxygen delivery and enzymatic activity[7]. As mentioned above, pregnant women require more iron nutrition than non-pregnant women, indicating that ID is one of the most common nutritional problems during pregnancy[3]. ID is the most common cause of anemia in developing countries[8]. With the enlargement of blood volume, pregnant women need more iron, especially those in the second trimester of pregnancy, than non-pregnant women[3]. Physiologically, the development of ID can be divided into three stages, depletion of iron stores, iron depletion erythropoiesis without anemia, and iron deficiency anemia[9]. Because the symptoms of the first two stages are often not obvious, people tend to overlook the effect of ID, which can easily develop ID anemia. Serum ferritin (SF) is one of the most important clinical indicators of ID and can reflect iron reserves in the body[4]. SF is a highly sensitive and specific biochemical marker for ID, because SF levels begin to decline before the onset of ID dysfunction.

Thyroid dysfunction is a common complication in pregnant women. The prevalence of subclinical hypothyroidism in pregnant women varies from 4% to 17%, and the positive rate of anti-thyroid peroxidase (anti-TPO) antibody in pregnancy varies from 5.1% to 12.4%[10]. Thyroid hormones play crucial roles in the process of human growth and development, especially in the early stage of brain development[11]. Previous human case reports have indicated that children are at high risk of severe physical and mental handicaps or problems if their mothers have severe hypothyroidism[12]. These studies highlight the value of maternal thyroid function in pregnancy and the effects of maternal thyroid diseases on fetal development. Among all of the systems affected by thyroid hormones, the central nervous system (CNS) is prominent due to its high sensitivity to thyroid hormones during development[13]. The patients with thyroid dysfunction have various clinical symptoms. Excess thyroid hormone results in tachycardia, anxiety, tremor of the extremities, and weight loss[14]. Hypothyroidism causes dry skin, developmental retardation, hearing and speech impairment, and the most serious symptom is cretinism, which could lead to dementia, mental retardation and psychomotor disorder[13, 15]. In general, thyroid hormone deficiency affects CNS through following ways: reduction of progenitor expansion, delay in neuron proliferation, and decreased expression of proteins involved in synaptic plasticity[13]. Therefore, thyroid hormone levels during pregnancy should be of particular concern.

In recent years, some studies have indicated that ID during pregnancy may affect the level of thyroid hormones, and thyroid function may also interact with iron status[16, 17]. Therefore, through the investigation of thyroid hormone, iron nutrition and anemia status of women in the pregnant metaphase in Pingguo county, this paper further explores the relationship among ID, anemia and thyroid function.

Materials and methods

Study site and subjects

A hospital-based case-control study was conducted in Pingguo County, Guangxi, China.

Pingguo County is located in in the southern of China, which is an iodine-sufficient area and a suitable research site for thyroid dysfunction.

Participants were recruited from the population of pregnant women during their routine inspection in Pingguo Maternal and Child Health Hospital from May 2017 to August 2018. The hospital provides service for pregnant women and children. This study was approved by the Medical Research Ethics Committee of the local Maternity and Child Health Hospital, and all the women involved in this study gave informed consent.

Pregnant women aged 18 - 46 years who lived in Pingguo for more than 6 months and only screened in the second trimester (gestational age between 14 and 27 weeks) were invited to participate in the study. The exclusion criteria were as follows: 1) pregnant women with a history of thyroid disease, such as Christine disease; 2) pregnant women with a family history of mental retardation or organic mental disorder and dementia, hereditary endocrine and metabolic diseases, cancer, congenital malformations, and developmental dysplasia; 3) pregnant women addicted to the drugs and/or alcohol; 4) pregnant women lived in Pingguo County less than 6 months; 5) pregnant women with communicable diseases, including HIV, tuberculosis and HBV; and 6) those withholding consent to participate. In this study, 550 eligible pregnant women (gestational age ranging between 14 and 27 weeks) were recruited from May 2017 to August 2018.

Blood sample collection

The venous blood was collected from each subject, and the blood samples were centrifuged to obtain serum and erythrocyte for laboratory testing or storage at -80 for further analysis.

Iron status assessment

Serum ferritin was measured by electrochemiluminescence immunoassay kits (Roche Diagnostics GmbH, Cobas e601 analyzer, Germany), and hemoglobin was measured by colorimetry. The levels of SF (reference range, 13.00 - 150.00 µg/L) and Hb (reference range, 110 - 150 g/L) were evaluated respectively. Based on the recommendation for diagnosis of ID in pregnancy[18], women who had serum ferritin levels < 20µg/L were classified as group ID.

Thyroid function testing

All serum acquired were immediately tested for T3, FT3, T4, FT4, and TSH levels on the same day using the commercial electrochemiluminescence immunoassay kits (Roche Diagnostics GmbH, Cobas e601 analyzer, Germany). The levels of T3 (reference range, 1.20 - 3.10 nmol/L), FT3 (reference range, 3.10 - 6.80 pmol/L), T4 (reference range, 66.00 - 181.00 nmol/L), FT4 (reference range, 9.87 - 17.26 pmol/L), and TSH (reference range, 0.39 - 5.22 µIU/ml) were determined on the same day.

Statistical analysis

According the concentration of serum ferritin, pregnant women were classified into two groups (ID group and Normal group). Statistical analysis was performed using the Statistical Product and Service Solutions software (SPSS, version 24.0, IBM SPSS Statistics, USA) and the R Programming Language (R, version 4.0.4, Ross Ihaka,Robert Gentleman). Kolmogolov-Smirnov and Shapiro-Wilk tests were used to analyze data distribution normality. All the subjects showed skewed distribution. Thus, each group of statistics were described with medians and percentile values. Then, nonparametric Mann-Whitney U test was used to compare the differences between the two groups. Spearman's rank correlation test was used to estimate the relationship between serum ferritin and thyroid heroines, hemoglobin, and thyroid heroines separately. The factors of iron nutritional status and thyroid heroines were used to analyze the canonical correlation to extract principal features through the correlation between two sets of variables to further explore the relationship between iron nutritional status and thyroid heroines. Elastic network regression was used to explore their specific relationship. The result was considered as statistically significant difference when the 2-tailed P-value was < 0.05.

Results

Characteristics of the subjects

In total, the mean age (±SD) was 29±6 years old, and the mean gestation age (±SD) was 20±4 weeks (550 cases). In this study, the median SF level was 35.63 µg/L (range, 7.14 - 253.49 µg/L), the median Hb level was 115.00 g/L (range, 91.78 - 133.00 g/L), the median T3 level was 2.36 nmol/L (range, 1.43 - 4.14 nmol/L), the median FT3 level was 4.25 pmol/L (range, 2.94 - 6.31 pmol/L), the median T4 level was 110.80nmol/L (range, 64.41 - 181.50 nmol/L), the median FT4 level was 13.49 pmol/L (range, 8.66 - 20.66 pmol/L), and the median TSH level was 1.42 µIU/ml (range, 0.01 - 5.28 µIU/ml), as shown in*Table 1* . The ID group was significantly lower in FT3 and FT4, whereas it was significantly higher in TSH (all P < 0.01). The results of the difference analysis of thyroid hormone levels between the two groups of the 550 cases are listed in *Table 1* and*Figure S1*.

Relationship between iron status and thyroid function

In this study, Spearman's rank correlation analysis revealed that both of SF and Hb were correlated with FT3, FT4, and TSH (all P < 0.01). Specifically, the results showed that the levels of SF were positively related to FT3 levels (r = 0.31, p < 0.01), T3 levels (r = 0.09, p < 0.05), FT4 levels (r = 0.28, p < 0.01), and T4 levels (r = 0.09, p < 0.05), and negatively related to TSH levels (r = -0.22, p < 0.01). In addition, as an anemia variable, Hb was involved in this statistical analysis. Hb was positively associated with the levels of T3, FT3, T4, and FT4, and negatively associated with TSH levels (all P < 0.01). *Table S1* ,*Figure 1* , and*Figure S2*summarized the correlation coefficients.

The canonical correlation analysis between iron nutrition and thyroid hormone

We performed canonical correction analysis to explore the structure of correlation by using a method similar to multivariate analysis. The set of iron nutrition was assigned as X variables (X1 = SF and X2 = Hb). Meanwhile, the set of thyroid hormones was assigned as Y variables (Y1 = T3, Y2 = FT3, Y3 = T4, Y4 = FT4, and Y5 = TSH). There were two canonical functions. However, only the first canonical function was statistically significant (canonical coefficient = 0.431, $P < 0.01$). On the base of standardized canonical coefficients between the canonical function and all variables (U in iron status and V in thyroid hormones), the equations could be established as follows:

U1= -0.648X1 - 0.591X2

V1= 0.388Y1 - 0.970Y2 + 0.135Y3 - 0.308Y4 + 0.213Y5

The canonical correlation analysis showed that a significant positive correlation existed between iron nutrition and thyroid hormone. The proportion of explaining the variance attributed to a given canonical were 96.22% in the 1st canonical function (*Table S2*). U1 and V1 explained 35.6% and 65.1% of iron nutritional status and thyroid hormone variables, respectively. As shown in the illustration, the structure coefficients pointed that except the variable of TSH, other variables had positive correlations with iron nutrition variances (*Fig. 2* and*Fig. S3*). Moreover, the standardized coefficients of SF and FT3 were the highest in their respective subsets. The larger the standardized canonical coefficients are, the greater the weight in their respective typical variables is. Consistent with the former, the standardized canonical coefficients of SF and FT3 were the highest in their respective subsets as well. We next performed elastic network regression due to the fact that coefficient of SF was almost the same as the Hb in our results.

The elastic network regression between iron nutrition and thyroid hormone

The elastic network regression was used to further explore of the specific relationship between iron nutrition and thyroid function. Based on the results of canonical correlation and Spearman correlation, we chose FT3 as the dependent variable, whereas other factors were the independent variables. In our study, all factors were incorporated into the elastic network regression forecasting model (Alpha = 0.1, Lambda = 0.0032) (*Fig. 3*), and the equations could be established as follows.

FT3 = 0.3802 + 0.7191T3 – 0.0022T4 + 0.1062FT4 – 0.0014TSH + 0.0014SF + 0.0077Hb

From the results of elastic network regression, we found that FT3 was influenced by T3, and the FT4 was the second; these findings were consistent with the simple correlation results in our study. However, they were inconsistent with the canonical correlation results. Thus, based on the above results, it was still impossible to get an exact result to prove whether SF or HB played a more important role in the thyroid function. It was also proved that iron nutrition affected thyroid function in many ways.

Discussion

Pregnant women in mid-pregnancy period are prone to ID[2]. Serum ferritin plays an important role in reflecting the iron reserves[4]. Our study showed significant correlations between thyroid hormones and iron status. Although there were some correlations between thyroid hormones and iron status factors, there were not completely consistent in a previous study. Shan et al. pointed out that ID women had a lower FT4 levels, but had no significant difference in TSH levels[19]. We did not have evidence that directly supported the specific relationship between thyroid hormones and iron nutrition. However, our results and those of previous studies indicated that close relationship between thyroid hormone and iron nutrition. Especially, our results can be considered as a certain reference due to be based on a large sample size.

In a previous study that established the trimester-specific reference interval of thyroid hormones in pregnant women, it was pointed out that the FT4 RI in the second trimester was 11.70 (8.67, 16.21) pmol / L, and the TSH RI in the second trimester was 1.64 (0.27, 4.53) µIU/mL[20]. The RIs of thyroid hormones in our study differed from those in previous reports because of differences in detection reagents, races, detection methods or kits,and individual differences. Compared with other studies, the reference critical values mentioned in our study were slighter higher. However, the IRs of each parameter established in this study had certain reference significance.

In recent years, the number of studies on the relationship between thyroid and iron nutrition increased. Some papers pointed out that the high level of TSH and the low level of FT4 were independently associated with ID in pregnant women[21]. Other studies also showed that SF was an important predicter of TSH during the second trimester of pregnancy[4]. ID might result in higher TSH and lower FT4, FT3 in the pregnancy, which affects the thyroid function of pregnant woman[22]. Some researchers found that ID decreases the level of SF, FT3, FT4, and enzyme activity of TPO in rats[23]. However, a study indicated no significant correlation was found between thyroid function and ferritin in pregnant women from Indonesia[24]. And a study pointed out that iron nutrition was positively correlated with FT4 during pregnancy, except in the third trimester[25]. In the current study, we observed that women in ID group who were assessed for SF levels during the second trimester pregnancy showed lower levels of FT3 and FT4 and a higher level of TSH. Our results were consistent with those of most previous studies. Therefore, comparison of the findings with other previous studies could confirm that the iron nutrition status was positively associated with thyroid hormones during the second trimester pregnancy. However, it is unclear how iron status interacts with thyroid functions specifically. Thus, further research is needed.

Although iron status is not the only factor affecting thyroid function, it has a significant auxiliary effect[4]. Several studies indicated that ID influenced thyroid function by the following mechanism. Some studies pointed out that the decline in FT4 concentrations might be associated with the change of the TPO activity; TPO is an iron-dependent enzyme that plays an important role in the production of thyroid hormones; therefore, ID might cause lower FT4 levels via reducing TPO activity; then, the levels of TSH increase in negative feedback[26-28]. Briefly, the lower FT4 levels secondary to ID results in the decrease in FT4 negative feedback inhibition of TSH levels, thereby increasing TSH levels[16].

When ID develops to some extent, it will result in ID anemia. Thus, as an anemia index, Hb is also involved in this study to compare the influence on thyroid function with SF (Hb $<$ 110g/L were found in 59% in ID group). Interestingly, Spearman correlation analysis showed that both SF and Hb positively correlated with FT4 and FT3 and negatively correlated with TSH. Yet canonical correlation analysis reflected the relationship between SF and FT3. Therefore, we were sure that iron nutritional status was associated with thyroid hormone, and we predicted SF and Hb were risk factors that contribute to thyroid hormones. Furthermore, as the results of canonical correlation contradicted with the results of regression, whether SF or Hb plays a key role in affecting the thyroid hormones is uncertain if our data analysis result is the only basis. Previous experiments suggested that Hb synthesis occurs prior to TPO when ID occurs[29]. Thus, we also speculated that when ID occurs, iron will be preferentially supplied to the maternal hemoglobin to maintain current levels, which results in lower SF levels and decreases synthesis and activity of TPO. Then, thyroxine insufficiency arises, especially the FT4 and FT3 levels. Lower FT3 and FT4 levels are obtained, thereby raising TSH levels via negative feedback inhibition.

Canonical correlation analysis is a useful statistical method to analyze the interrelationships between subsets of multiple dependent and independent variable quantities[30]. It could be used to analyze the complex interactions of date from two subsets of variable quantities[31]. In our study, to reduce the influence of collinearity and highlight the main relationship in two sets, canonical correction analysis was added. Considering that the units of variables studied were different, the standardized correlation coefficients were used. The larger the standardization coefficient was, the greater the weight reflected in typical variables in its subsets. The fact that both the standardized and the structure coefficients of SF and FT3 were the highest in their respective subsets reflected the relationship between SF and thyroid functions. SF was positively correlated with FT3 and FT4 and negatively correlated with TSH. In addition, the results of standardization coefficient and structure coefficient were consistent in our study.

The elastic network model is one of the most important models on nonlinear prediction. This model is controlled by two parameters, namely, Alpha and Lambda[32]. Being different from ridge and lasso regression, the elastic network model merges both regularization skills within the one model; thus, it not merely lessens the feature coefficients but also installs some of the coefficients to zero to reduce the dimensionality of the feature space; then, the potentially meaningful correlation structure also could be preserved[32, 33]. In our study, none of the included variables were excluded, and the coefficient of SF is slightly smaller than that of Hb. The results of elastic network contradicted with the results of canonical correlation, maybe because both SF and Hb played certain roles in regulating thyroid function. However, they are all indicators of iron nutrition. Thus, iron nutritional status is associated with thyroid hormone levels in the second trimester of pregnancy.

The innovations in our study were pointed out as follows. First of all, our result was based on larger samples from the same regions, and all of recruited women were in the second trimester of pregnancy. Throughout the whole pregnancy, the thyroxin level in serum is fluctuating. For example, due to the change of human chorionic gonadotropin, the level of FT4 elevated temporarily and then declined gradually[34]. To our knowledge, this is the first study to compare the influence of SF and Hb on thyroid hormones via canonical correlation and elastic network regression analysis. Our study had some limitations. Firstly, our study was based on the results of large population survey. Thus, there was a lack of experimental for further demonstrations. However, the change tendency of our results was consistent with previous studies and experiments. Then, we did not measure the levels of urine iodine or thyroid antibodies. Thus, we could not perform a comprehensive evaluation of pregnant women. Nevertheless, it will be beneficial to perform similar studies in different trimesters of pregnancy to do further study the relationship between iron nutritional status and thyroid function.

Conclusion

In conclusion, this study provides the data on iron nutritional status and thyroid hormones of pregnant second-trimester women in Pingguo County. During the second trimester of pregnancy, ID may result in lower FT4 and FT3 and higher TSH, and these findings are consistent with the earlier reports. Indeed, SF is positively correlated with FT3 and FT4 levels, and negatively correlated with TSH levels. Hb may also play a certain role in affecting the thyroid function. Although the specific mechanism is still unclear, the correlation between iron nutrition and thyroid hormone should not be underestimated. Involving two common endocrine diseases in pregnancy, the relationship between ID and thyroid hormone, the molecular mechanisms involved, and the disease consequences deserve further investigation. From the perspective of maintaining maternal health and offspring's health, we recommend that ID should be estimated and treated to prevent thyroid dysfunction during the second trimester of pregnancy or even earlier. In addition, it also provides a new idea and reference for improving the quality of life of pregnant women and their offspring.

Disclosure of interests

No conflict of interest. Completed disclosure of interest forms are available to view online as supporting information.

Contribution to authorship

DPH, LY, FYW, and YXZ conceived and designed this study. FYW and YXZ were responsible for writing the manuscript draft. DPH and LY revised substantially to the final manuscript. FYW and ZXY contributed to data analysis, YNL offered suggestions in the data analysis,and SL contributed the literature research. XYZ and XQQ provided guidance in the investigation. FYW, YXZ, DPH, LY, ZXY, XYZ and XQQ contributed to data interpretation. FYW and YXZ contributed equally to this article. DPH and LY contributed equally to this article. All authors approved the final manuscript.

Details of ethic approval

The study was approved by the Guangxi Medical University Ethics Committee ((2018) No.071) on March 6, 2018.

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References

1. Christian B, Michael A. Iron deficiency in gynecology and obstetrics: clinical implications and management. Hematology American Society of Hematology Education Program. 2017;2017(1).

2. Zuguo M, E CM, C LA, M PC, E CS, A LD, et al. Assessment of iron status in US pregnant women from the National Health and Nutrition Examination Survey (NHANES), 1999–2006. Mei Zuguo;Cogswell Mary E;Looker Anne C;Pfeiffer Christine M;Cusick Sarah E;Lacher David A;Grummer-Strawn Laurence M. 2011;93(6).

3. O ST. Iron status during pregnancy: setting the stage for mother and infant. The American journal of clinical nutrition. 2005;81(5).

4. Leqi H, Chunmei S, Yanan Z, Zaoping C, Heyuan D, Jun L, et al. Evaluation of serum ferritin and thyroid function in the second trimester of pregnancy. Endocrine journal. 2018;65(1).

5. W BT, R PJ, K GM, W AG. Perinatal iron and copper deficiencies alter neonatal rat circulating and brain thyroid hormone concentrations. Endocrinology. 2010;151(8).

6. W BT, C LK, W AG, R PJ. Maternal iron supplementation attenuates the impact of perinatal copper deficiency but does not eliminate hypotriiodothyroninemia nor impaired sensorimotor development. The Journal of nutritional biochemistry. 2011;22(11).

7. K GM. Iron deficiency in pregnancy. American journal of obstetrics and gynecology. 2020;223(4).

8. Zimmermann MB, Hurrell RF. Nutritional iron deficiency. The Lancet. 2007;370(9586).

9. Breymann C. Iron Deficiency Anemia in Pregnancy. Seminars in Hematology. 2015;52(4).

10. Springer D, Jiskra J, Limanova Z, Zima T, Potlukova E. Thyroid in pregnancy: From physiology to screening. Critical Reviews in Clinical Laboratory Sciences. 2017;54(2).

11. Bernal J. Thyroid hormone regulated genes in cerebral cortex development. Journal of Endocrinology. 2017;232(2).

12. Linding AS, Allan C, Jesper K, Bülow PI, Stig A. MECHANISMS IN ENDOCRINOLOGY: Neurodevelopmental disorders in children born to mothers with thyroid dysfunction: evidence of fetal programming? European journal of endocrinology. 2017;177(1).

13. Giovanni P, Cosimo G, Francesco C. Effect of Thyroid Hormones on Neurons and Neurodevelopment. Hormone research in paediatrics. 2018.

14. De Leo S, Lee SY, Braverman LE. Hyperthyroidism. The Lancet. 2016;388(10047):906-18.

15. Chaker L, Bianco AC, Jonklaas J, Peeters RP. Hypothyroidism. The Lancet. 2017;390(10101):1550-62.

16. Zhang Y, Huang X, Chen Z, Yang Q, Li X, Zhang R, et al. Iron Deficiency, a Risk Factor for Thyroid Autoimmunity During Second Trimester of Pregnancy in China. Endocrine Practice. 2020;26(6).

17. Khatiwada S, Gelal B, Baral N, Lamsal M. Association between iron status and thyroid function in Nepalese children. Thyroid Research. 2016;9(1).

18. Branch CMAPM. Guidelines for diagnosis and treatment of gestational iron deficiency and iron deficiency anemia. Chinese Journal of Perinatal Medicine 2014:451-4.

19. Zhongyan S, Lulu C, Xiaolan L, Chao L, Bingyin S, Lixin S, et al. Iodine Status and Prevalence of Thyroid Disorders After Introduction of Mandatory Universal Salt Iodization for 16 Years in China: A Cross-Sectional Study in 10 Cities. Thyroid : official journal of the American Thyroid Association. 2016;26(8).

20. Xing J, Yuan E, Li J, Zhang Y, Meng X, Zhang X, et al. Trimester- and Assay-Specific Thyroid Reference Intervals for Pregnant Women in China. International Journal of Endocrinology. 2016;2016.

21. B ZM, Hans B, F HR. Iron deficiency predicts poor maternal thyroid status during pregnancy. Pubmed. 2007;92(9).

22. Fu J, Yang A, Zhao J, Zhu Y, Gu Y, Xu Y, et al. The relationship between iron level and thyroid function during the first trimester of pregnancy: A cross-sectional study in Wuxi, China. Journal of Trace Elements in Medicine and Biology. 2017;43.

23. Y HS, B ZM, Myrtha A, Wolfgang L, F HR. Iron deficiency anemia reduces thyroid peroxidase activity in rats. The Journal of nutrition. 2002;132(7).

24. SUPADMI S, KUSRINI I, KUSUMAWARDANI HD. The Influence of Iron Depletion and Chronic Energy Deficiency on the Risk of Hypothyroidism in Pregnant Women Living in Iodine Deficiency Disorders Endemic Areas in Badegan Ponorogo District East Java, Indonesia:Public Health Nutrition. Journal of Nutritional Science and Vitaminology. 2020;66(Supplement).

25. Xiaochun T, Zhongyan S, Chenyan L, Xiaohui Y, Jinyuan M, Weiwei W, et al. Iron Deficiency May Predict Greater Risk for Hypothyroxinemia: A Retrospective Cohort Study of Pregnant Women in China. Thyroid : official journal of the American Thyroid Association. 2018.

26. L BJ, E BD, K KS, H GM. Plasma thyroid hormone kinetics are altered in iron-deficient rats. The Journal of nutrition. 1998;128(8).

27. M. SS, E. JP, C. LH. In vitro hepatic thyroid hormone deiodination in iron-deficient rats: Effect of dietary fat. Smith Scott M;Johnson Phyllis E;Lukaski Henry C. 1993;53(8).

28. I SM. Effect of thyrotropin on thyroidal iodine metabolism during hypoxia. The American journal of physiology. 1969;216(2).

29. Xiaona H, Xiaochun T, Hongzhi Z, Zhongyan S, Jing L, Ting J, et al. Iron deficiency without anemia causes maternal hypothyroxinemia in pregnant rats. Nutrition research (New York, NY). 2014;34(7).

30. Kyungmin K, Woo KG, Jungmin W. The Quality of Life of Hemodialysis Patients Is Affected Not Only by Medical but also Psychosocial Factors: a Canonical Correlation Study. Journal of Korean medical science. 2018;33(14).

31. Kowalski J, Tu XM, Jia G, Perlis M, Frank E, Crits-Christoph P, et al. Generalized covariance-adjusted canonical correlation analysis with application to psychiatry. Statistics in Medicine. 2003;22(4).

32. Teipel SJ, Grothe MJ, Metzger CD, Grimmer T, Sorg C, Ewers M, et al. Robust Detection of Impaired Resting State Functional Connectivity Networks in Alzheimer's Disease Using Elastic Net Regularized Regression. Front Aging Neurosci. 2016;8:318.

33. Jerome F, Trevor H, Rob T. Regularization Paths for Generalized Linear Models via Coordinate Descent. Journal of statistical software. 2010;33(1).

34. Mariacarla M, Francesco T, Francesco V. Thyroid physiology in pregnancy. Endocrine practice : official journal of the American College of Endocrinology and the American Association of Clinical Endocrinologists. 2014;20(6).

Table 1 Demographic characteristics, thyroid hormones, and iron nutrition of pregnant women during the second trimester

	Total	**Total**	**Iron Deficiency group**	**Iron Deficie**
Number of cases	550	550	193	193
General characteristics	**General characteristics**			
Age#	29±6	29±6	28±6	28±6
Gestational weeks#	20±4	20±4	23±3	23±3
Thyroid hormone				
T3 (nmol/L)*	2.36 (1.43, 4.14)	2.36 (1.43, 4.14)	2.33 (1.37, 4.21)	2.33 (1.37, 4.2
FT3 (pmol/L)*	4.25 (2.94, 6.31)	4.25 (2.94, 6.31)	3.91 (2.64, 6.08)	3.91 (2.64, 6.0
T4 (nmol/L)*	110.80 (64.41, 181.50)	110.80 (64.41, 181.50)	109.50 (59.94, 173.84)	109.50 (59.94,
FT4 (pmol/L)*	13.49 (8.66, 20.66)	13.49 (8.66, 20.66)	12.49 (8.60, 18.61)	12.49 (8.60, 1
TΣH (μIΥ/μλ)*	1.42 (0.01, 5.28)	1.42 (0.01, 5.28)	1.64 (0.02, 5.98)	1.64 (0.02, 5.9
Iron nutrition				
ΣΦ (μγ/Λ)*	35.63 (7.14, 253.49)	35.63 (7.14, 253.49)	11.72 (5.28, 19.30)	11.72 (5.28, 1
Hb (g/L)*	115.00 (91.78, 133.00)	115.00 (91.78, 133.00)	108.00 (86.70, 127.30)	108.00 (86.70,

Note: P -values: iron deficiency group vs. normal group; $P < 0.05$ was considered to be statistically significant.

Means ± SD

*Medians (the 2.5th percentile, 97.5th percentile)

Table S1 Relationship between iron status and thyroid function in the second trimester

		T3	**FT3**	**T4**	**FT4**	**TSH**
SF	r	0.09	0.31	0.09	0.28	-0.21
	p	0.03	0.00	0.04	0.00	0.00
Hb	r	0.16	0.34	0.14	0.30	-0.16
	p	0.00	0.00	0.00	0.00	0.00

Note: P -values and correlation coefficient r were calculated using Spearman's rank correlation, and $P < 0.05$ was considered to be statistically significant.

Table S2 Canonical correction between iron nutritional status and thyroid hormones

Canonical function	Canonical correlation	Eigen value	Wilk's λ	proportion	*P*
First	0.43	0.23	0.807	0.96	0.00
second	0.09	0.01	0.991	0.04	0.31

Note: $P < 0.05$ was considered to be statically significant.

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`tables and figures.docx` available at https://authorea.com/users/734346/articles/711418-iron-nutritional-status-is-associated-with-thyroid-hormone-levels-in-the-second-trimester-of-pregnancy-a-case-control-study