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ORIGINAL ARTICLE

Association of ABO and RhD Blood Types with Thyroid Abnormalities in Pregnant Women

Jiahong Chen *, Hongtao Jiao *, Jing Liu, Mingming Wang, Ning Zhang, Kun Chi, Hui Liu, Jinlian Song, Peng Sun, Yanzhen Wan

* *Jiahong Chen and Hongtao Jiao contributed equally to the study*
Clinical Laboratory, Women and Children's Hospital, Qingdao University, Qingdao, China

ABSTRACT

Background: **The associations between the blood group system and various diseases have long been investigated since the discovery of blood group antigens. However, up to now, the relationship of ABO and RhD blood types with thyroid abnormalities in pregnant women has not been reported yet. The present study aims to investigate the association of ABO and RhD blood types with thyroid abnormalities in pregnant women in Qingdao, China.**
Methods: **A total of 5,675 pregnant women aged 18 - 50 years old at 7 to 13 weeks of gestation were enrolled into this cross-sectional study. Serum concentrations of free T3 (FT3), free T4 (FT4), thyroid stimulating hormone (TSH), total T3, total T4, thyroid peroxidase antibody (TPOAb), thyroglobulin antibody (TGAb), thyrotrophin receptor antibody (TRAb), ferritin (FERR), and vitamin B12 were detected, and blood type identification was performed. The association of ABO and RhD blood types with thyroid abnormalities was evaluated by logistic regression analysis.**
Results: **Pregnant women with blood type A and B had significantly higher concentrations of TSH than women with blood type O, and FERR and B12 levels were remarkably increased in RhD(+) women than in RhD(-) women. Pregnant women with blood type A were less likely to be TRAb(+), and pregnant women with blood type AB were more likely to suffer from clinical or subclinical hypothyroidism, compared with blood type O. In addition, RhD(+) pregnant women had less likelihood to be TRAb(+) in comparison to RhD(-) women.**
Conclusions: **Our findings indicate novel meaningful correlations between ABO and RhD blood types and thyroid disorders in pregnant women, which will offer guidance for better screening and management of thyroid function in pregnant women.**
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Correspondence:
Yanzhen Wan
Clinical Laboratory, Women and Children's Hospital
Qingdao University
No. 6 Tongfu Road
Shibei District
266034, Qingdao
China
Email: wanyanzhen1979@163.com

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INTRODUCTION

Thyroid abnormalities are prevalent in pregnant women. Normal thyroid function during gestation is of great importance for both maternal health and fetal development, and thyroid disorders can lead to adverse obstetric outcomes [1]. Hyperthyroidism is mainly caused by Graves' disease and gestational transient thyrotoxicosis (GTT) in pregnant women [2]. Graves' disease, as a

kind of autoimmune thyroid diseases (AITD), is believed to be mediated by autoimmunity to the thyrotropin receptor. Thyrotropin receptor antibody (TRAb) is an effective biomarker for diagnosis and treatment of Graves' disease. Clinical hyperthyroidism during pregnancy is related with maternal and fetal complications such as abortion, pre-eclampsia, maternal and fetal congestive heart failure, preterm delivery, maternal thyroid storm, intrauterine growth retardation, and fetal and/or neonatal hyperthyroidism, etc. [3]. GTT is caused by high concentrations of human chorionic gonadotropin (HCG) due to structural homology between HCG and thyrotropin, which is not associated with adverse gestational outcomes. Autoimmune thyroiditis and iodine deficiency are the two most frequent causes of hypothyroidism during pregnancy. The former, as a common AITD, is prevalent in general population and can present with normal or abnormal thyroid function. Thyroid peroxidase antibody (TPOAb) and thyroglobulin antibody (TGAb) are frequently elevated and viewed as biomarkers of autoimmune thyroiditis [4]. Adverse outcomes of overt hypothyroidism during pregnancy have been well documented. It is commonly associated with adverse offspring and obstetric outcomes including gestational diabetes mellitus, pregnancy loss, pre-eclampsia, preterm labor, infant intensive care unit admission, low birth weight and adverse fetal and/or child neurodevelopment [1,5,6]. However, the effects of maternal subclinical hypothyroidism and autoimmune thyroiditis with euthyroidism on obstetric outcomes remain controversial [4,7,8]. Therefore, appropriate management of thyroid disorders in pregnancy is pivotal to the health of both pregnant women and their offspring.

The blood group antigens of ABO and RhD system play a vital role in transfusion medicine. Besides this, ABO blood groups have been demonstrated to be associated with host susceptibility to various infectious and noninfectious diseases, including cardiovascular diseases (myocardial infarction, cerebral ischemic events, etc.), some types of cancers (gastric cancer, thyroid cancer, etc.), metabolic diseases, cognitive disorders, virus infections (corona virus disease 2019, mumps, etc.), bacterial infections (tuberculosis, pseudomonas aeruginosa infection, etc.), and parasite infections (malaria, cholera, etc.) [9,10]. The RhD antigen has been mainly linked to hemolytic disease of the fetus and newborn, and beyond this direct effect little is known about its relationship with disease development or pathogenesis [11].

Reports about the association of ABO and RhD blood types with thyroid abnormalities are scarce. Tam AA et al. reported that thyroid cancer patients with blood type B were in higher risk of extrathyroidal extension and advanced stage compared to patients with non-B [12], while Dogan O found that development of papillary thyroid cancer (PTC) was not associated with ABO/Rh blood groups, and there was no significant difference of the clinical and pathological characteristics of PTC patients according to blood type distribution [13]. In addition, another study showed that blood type O was more frequently observed among patients with Hashimoto's thyroiditis-induced hypothyroidism, compared with other benign thyroid diseases [14]. However, to the best of our knowledge, there is no report about the association of ABO and RhD blood types with thyroid abnormalities in pregnant women yet. So, our study is aimed to investigate the distribution of ABO and RhD blood types in pregnant women with different thyroid abnormalities, compared with normal control, and to further explore the possible associations.

MATERIALS AND METHODS

Study design and participants

This was a cross-sectional study. A total of 5,675 pregnant women aged 18 - 50 years old at 7 to 13 weeks of gestation were enrolled into this study during the establishment of health archive of pregnancy in Qingdao Women and Children's Hospital Affiliated to Qingdao University from March 2023 to December 2023. This hospital is the biggest specialized center for gynecology, obstetrics and pediatrics in Qingdao district, China. Basic information such as out-patient number, name, age, and gestational weeks was collected and recorded.

Laboratory testing of parameters associated with thyroid function

Venus blood samples of every participant were collected and serum was separated and stored at -80°C for future usage. Concentrations of free T3 (FT3), free T4 (FT4), thyroid stimulating hormone (TSH), total T3 (TT3), total T4 (TT4), thyroid peroxidase antibody (TPOAb), thyroglobulin antibody (TGAb), thyrotrophin receptor antibody (TRAb), ferritin (FERR), and vitamin B12 were designed to be detected. Serum FT3, FT4, TSH, TPOAb, and TGAb levels were detected with the chemiluminescent method using ADVIA Centaur XP Immunoassay System (SIEMENS), and levels of TT3, TT4, TRAb, FERR, and B12 were detected with the electrochemiluminescent method using Cobas e801 immunoassay analyzer (Roche). TPOAb > 60.0 IU/mL, TgAb > 4.5 IU/mL and TRAb > 1.75 IU/L were considered to be positive, according to the reagent instructions. A total of 629 cases with singleton pregnancy were included to establish the specific reference range of TSH at the first-trimester gestation, excluding those with positive TPOAb, TGAb or TRAb, those on medication, and those with medical history of thyroid disorders or autoimmune diseases. TSH level was not in accordance with normal distribution, so a nonparametric methodology was used to calculate the reference range. The 2.5th and 97.5th percentile was defined as the lower and upper limit of the reference range, respectively. The reference range for serum TSH was established as 0.040 - 3.168 mIU/L in the study.

Identification of ABO and RhD blood types

Whole blood samples were collected in vacuum blood collecting tubes with anticoagulant EDTA-K2 for blood

type identification. ABO and RhD blood types were identified using ABO & RhD blood grouping cards with Microcolumn Gel Immunoassay (Changchun Bo Xun Biotechnology, LLC, China).

Grouping of the participants according to thyroid status

Participants who were negative for TPOAb, TGAb, and TRAb, and had a TSH value between 0.040 and 3.168 mIU/L were included in the control group. Those who were positive for TPOAb were classified into the TPOAb(+) group, those with positive TGAb into the TGAb(+) group, those with positive TPOAb or positive TGAb into the TPOAb(+) or TGAb(+) group, and those with positive TRAb into the TRAb(+) group. In addition, those participants with a TSH value higher than 3.168 mIU/L were categorized into the clinical or subclinical hypothyroidism group, and those with a TSH value lower than 0.040 mIU/L into the clinical or subclinical hyperthyroidism group.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics 27. For continuous data, normal distribution was first performed. Data in accordance with normal distribution were expressed as mean ± standard deviation. Otherwise, data were expressed as median (Interquantile Range) (Md (P25, P75)). Comparison among different ABO blood types was performed with Kruskal-Wallis test, and comparison between RhD(-) and RhD(+) was performed with Mann-Whitney U test. For categorical variables, data were expressed as number (percentage) and compared by chi-squared test. The association of ABO and RhD blood types with thyroid abnormalities was evaluated by logistic regression analysis. Odds ratio (OR) and adjusted odds ratio (aOR) with 95% confidence interval (95% CI) were calculated. A probability value of < 0.05 was considered to be statistically significant.

RESULTS

Age distribution in different groups of thyroid abnormalities

As is shown in Table 1, age distribution of the TPOAb(+) group was significantly different from that of the control group ($p < 0.001$), so was the TGAb(+) group ($p = 0.01$), the TPOAb(+) or TGAb(+) ($p < 0.001$) group, and the TRAb(+) group ($p < 0.001$). To investigate the relationship between maternal age and different thyroid abnormalities, logistic regression analysis was performed and results showed that older age was significantly associated with the likelihood of TPOAb(+), TGAb(+), TPOAb(+) or TGAb(+), and TRAb(+), and the older maternal age was, the higher the risk of suffering from TPOAb(+), TGAb(+), TPOAb(+) or TGAb(+), and TRAb(+) became (Table 2). For the TPOAb(+) group, compared with the aged < 30 group, OR (95% CI) for aged 30 - 34 and aged ≥ 35 was 1.257 (1.077, 1.466) and 1.429 (1.198, 1.706), respectively. For the TGAb(+) group, OR (95% CI) was 1.221 (0.991, 1.504) and 1.430 (1.129, 1.812), respectively. For the TPOAb(+) or TGAb(+) group, OR (95% CI) was 1.260 (1.087, 1.460) and 1.454 (1.229, 1.722), respectively. And for the TRAb(+) group, OR (95% CI) was 2.136 (1.339, 3.407) and 2.431 (1.455, 4.062), respectively (Table 2).

Distribution of ABO and RhD blood types in different groups of thyroid abnormalities

ABO distribution in the control group was as follows: O (28.5%), A (28.2%), B (32.7%), and AB (10.6%), which was consistent with that reported for the local population. ABO distribution in the clinical or subclinical hypothyroidism group was O (26.2%), A (29.1%), B (26.2%), and AB (18.6%), which was significantly different from that of the control group ($p = 0.008$). Blood type AB was more likely to be observed in the clinical or subclinical hypothyroidism group. No significant difference was observed between the other groups and the control group. As for RhD status, RhD distribution in the control group was RhD(-) (0.5%) and RhD(+) (99.5%), while the distribution in the TRAb(+) group was RhD(-) (2.6%) and RhD(+) (97.4%), which was drastically different from that of the former ($p = 0.031$). RhD(-) was more likely to be observed in the TRAb(+) group. And there was no significant difference between the other groups and the control group (Table 3).

Comparison of different parameters associated with thyroid function among ABO and RhD blood types

As is demonstrated in Table 4, different parameters including FT3, FT4, TSH, TT3, TT4, TPOAb, TGAb, TRAb, FERR, and B12 were detected. Results showed that TSH level was remarkably different among ABO blood types. Md (P25, P75) for O, A, B and AB was 1.08 (0.56, 1.71), 1.19 (0.64, 1.92), 1.15 (0.62, 1.82), and 1.16 (0.67, 1.94), respectively ($p < 0.001$). TSH level in pregnant women with blood type A and B was significantly higher than that of women with blood type O (A vs. O, $p = 0.002$; B vs. O, $p = 0.005$). And no difference was observed for other parameters. As regards RhD blood types, FERR, and B12 levels were drastically higher in RhD(+) than in RhD(-) women. Md (P25, P75) of FERR for RhD(-) and RhD(+) was 55.02 (37.81, 73.12) and 73.30 (43.62, 115.50), respectively ($p = 0.029$). And Md (P25, P75) of B12 for RhD(-) and RhD(+) was 396.50 (326.90, 574.00) and 510.05 (398.20, 647.70), respectively ($p = 0.02$). There was no significant difference for other parameters.

Association of ABO and RhD blood types with different thyroid abnormalities

To further investigate the association of ABO and RhD blood types with different thyroid abnormalities, we performed logistic regression analysis. Table 5 showed that compared with blood type O, pregnant women with

Table 1. Age distribution of different groups.

	Control (TPOAb ≤ 60.0, TGAb ≤ 4.5, TRAb ≤ 1.75, 0.04 ≤ TSH ≤ 3.54) (n = 3,364)	TPOAb(+) (TPOAb > 60.0) (n = 1,155)	TGAb(+) (TGAb > 4.5) (n = 549)	TPOAb(+) or TGAb(+) (TPOAb > 60.0 or TGAb > 4.5) (n = 1306)	TRAb(+) (TRAb > 1.75) (n = 116)	Clinical or subclinical hypothyroidism (TSH > 3.168) (n = 172)	Clinical or subclinical hyperthyroidism (TSH < 0.040) (n = 270)
Maternal age	31 (28, 34)	31 (28, 35)	31 (28, 35)	31 (28, 35)	33 (30, 35)	30 (27, 34)	31.5 (28, 35)
p-value vs. control		< 0.001	0.003	< 0.001	< 0.001	0.198	0.042
< 30	1,361 (40.5%)	393 (34.0%)	189 (34.4%)	442 (33.8%)	27 (23.3%)	79 (45.9%)	97 (35.9%)
30 - 34	1,298 (38.6%)	471 (40.8%)	220 (40.1%)	531 (40.7%)	55 (47.4%)	60 (34.9%)	105 (38.9%)
≥ 35	705 (21.0%)	291 (25.2%)	140 (25.5%)	333 (25.5%)	34 (29.3%)	33 (19.2%)	68 (25.2%)
p-value vs. control		< 0.001	0.01	< 0.001	< 0.001	0.362	0.184

Maternal age was not in accordance with normal distribution, so continuous data were expressed as Md (P25, P75).

Table 2. The association between maternal age and different thyroid abnormalities.

	TPOAb(+) (n = 1,155)	TGAb(+) (n = 549)	TPOAb(+) or TGAb(+) (n = 1,306)	TRAb(+) (n = 116)	Clinical or subclinical hypothyroidism (n = 172)	Clinical or subclinical hyperthyroidism (n = 270)
	OR (95% CI) p	OR (95% CI) p	OR (95% CI) p	OR (95% CI) p	OR (95% CI) p	OR (95% CI) p
< 30	1.000	1.000	1.000	1.000	1.000	1.000
30 - 34	1.257 (1.077, 1.466) 0.004	1.221 (0.991, 1.504) 0.061	1.260 (1.087, 1.460) 0.002	2.136 (1.339, 3.407) 0.001	0.796 (0.564, 1.123) 0.195	1.135 (0.852, 1.511) 0.386
≥ 35	1.429 (1.198, 1.706) 0.000	1.430 (1.129, 1.812) 0.003	1.454 (1.229, 1.722) 0.000	2.431 (1.455, 4.062) 0.001	0.806 (0.532, 1.223) 0.311	1.353 (0.980, 1.869) 0.066

blood type A were less likely to be TRAb(+), OR (95% CI), 0.592 (0.355, 0.987), and pregnant women with blood type AB were more likely to suffer from clinical or subclinical hypothyroidism, OR (95% CI), 1.903 (1.190, 3.042)). In addition, pregnant women with RhD(+) would suffer from TRAb(+) less frequently compared with RhD(-) women, OR (95% CI), 0.203 (0.059, 0.698). Considering that maternal age was closely associated with the likelihood of TPOAb(+), TGAb(+), TPOAb(+) or TGAb(+), and TRAb(+), age was added as an independent variable. As is shown in Table 6, after adjustment for maternal age, the relationships persisted. The aOR (95% CI) for blood type A with TRAb(+), blood type AB with clinical or subclinical hypothyroidism, and RhD(+) with TRAb(+) was 0.593 (0.355, 0.990), 1.902 (1.189, 3.042) and 0.201 (0.058, 0.701), respectively.

DISCUSSION

Since the discovery of the ABO and RhD blood group systems, amounting researches have been conducted to investigate the associations between different blood types and various infectious or non-infectious diseases [11]. Thyroid disorders are common in pregnant wom-

Table 3. Distribution of ABO and RhD blood types in different groups.

	Control (n = 3,364)	TPOAb(+) (n = 1,155)	TGAb(+) (n = 549)	TPOAb(+) or TGAb(+) (n = 1,306)	TRAb(+) (n = 116)	Clinical or subclinical hypothyroidism (n = 172)	Clinical or subclinical hyperthyroidism (n = 270)
O (n (%))	958 (28.5%)	348 (30.1%)	168 (30.6%)	382 (29.2%)	41 (35.3%)	45 (26.2%)	90 (33.3%)
A (n (%))	948 (28.2%)	320 (27.7%)	168 (30.6%)	374 (28.6%)	24 (20.7%)	50 (29.1%)	75 (27.8%)
B (n (%))	1,100 (32.7%)	357 (30.9%)	158 (28.8%)	402 (30.8%)	40 (34.5%)	45 (26.2%)	79 (29.3%)
AB (n (%))	358 (10.6%)	130 (11.3%)	55 (10.0%)	148 (11.3%)	11 (9.5%)	32 (18.6%)	26 (9.6%)
p-value vs. control		0.565	0.247	0.625	0.226	<u>0.008</u>	0.360
RhD (-) (n (%))	18 (0.5%)	4 (0.3%)	2 (0.4%)	4 (0.3%)	3 (2.6%)	0 (0.0%)	0 (0.0%)
RhD (+) (n (%))	3,346 (99.5%)	1,151 (99.7%)	547 (99.6%)	1,302 (99.7%)	113 (97.4%)	172 (100.0%)	270 (100.0%)
p-value vs. control		0.427	0.586	0.305	<u>0.031</u>	1.000	0.228

Significant difference was underlined font.

Table 4. Comparison of different parameters associated with thyroid function among ABO and RhD blood types.

	O (n = 1,634)	A (n = 1,614)	B (n = 1,809)	AB (n = 618)	p-value	RhD(-) (n = 29)	RhD(+) (n = 5,646)	p-value
FT3 (pmol/L)	4.71 (4.39, 5.10)	4.70 (4.36, 5.04)	4.69 (4.365, 5.02)	4.68 (4.38, 5.00)	0.166	4.70 (4.28, 4.99)	4.70 (4.37, 5.05)	0.570
FT4 (pmol/L)	14.94 (13.70, 16.42)	15.045 (13.83, 16.425)	14.92 (13.685, 16.30)	14.975 (13.76, 16.43)	0.566	14.64 (13.38, 15.37)	14.97 (13.75, 16.37)	0.099
TSH (mIU/L)	1.08 (0.56, 1.71)	1.19 (0.64, 1.92)	1.15 (0.62, 1.82)	1.16 (0.67, 1.94)	<u>< 0.001</u>	1.17 (0.745, 1.89)	1.14 (0.61,1.83)	0.770
TT3 (nmol/L)	2.41 (2.13, 2.76)	2.39 (2.10, 2.73)	2.40 (2.11, 2.71)	2.42 (2.12, 2.78)	0.409	2.41 (2.21, 2.74)	2.40 (2.12,2.74)	0.998
TT4 (nmol/L)	146.30 (129.00, 163.90)	145.20 (129.00, 162.00)	143.40 (128.20, 161.10)	146.20 (128.00, 163.10)	0.242	143.35 (131.73, 161.50)	145.00 (128.90, 162.30)	0.820
TPOAb (IU/mL)	40.95 (29.50, 57.15)	40.10 (29.00, 55.70)	39.80 (29.40, 54.70)	41.20 (30.125, 56.75)	0.208	42.70 (34.95, 56.40)	40.40 (29.40, 55.90)	0.509
TGAb (IU/mL)	1.30 (1.30, 1.30)	1.30 (1.30, 1.30)	1.30 (1.30, 1.30)	1.30 (1.30, 1.30)	0.961	1.30 (1.30, 1.30)	1.30 (1.30, 1.30)	0.237
TRAb (IU/L)	0.80 (0.80, 0.96)	0.80 (0.80, 0.96)	0.80 (0.80, 0.99)	0.80 (0.80, 0.95)	0.611	0.81 (0.80, 1.00)	0.80 (0.80 ,0.97)	0.171
FERR (ng/mL)	73.20 (44.20, 117.00)	74.25 (43.50, 115.00)	72.57 (42.36, 115.13)	70.48 (43.76, 109.70)	0.460	55.02 (37.81, 73.12)	73.30 (43.62, 115.50)	<u>0.029</u>
B12 (pg/mL)	501.20 (383.63, 630.68)	513.60 (396.80, 656.00)	510.00 (404.20, 649.10)	523.35 (405.00, 659.60)	0.121	396.50 (326.90, 574.00)	510.05 (398.20, 647.70)	<u>0.020</u>

All parameters were not in accordance with normal distribution, so data were expressed as Md (P25, P75).
Significant difference was underlined font.

Table 5. The association of ABO and RhD blood types with different thyroid abnormalities (crude OR).

	TPOAb(+) (n = 1,155)	TGAb(+) (n = 549)	TPOAb(+) or TGAb(+) (n = 1,306)	TRAb(+) (n = 116)	Clinical or subclinical hypothyroidism (n = 172)	Clinical or subclinical hyperthyroidism (n = 270)
	OR (95% CI) p	OR (95% CI) p	OR (95% CI) p	OR (95% CI) p	OR (95% CI) p	OR (95% CI) p
O	1.000	1.000	1.000	1.000	1.000	1.000
A	0.929 (0.779, 1.108) 0.415	1.011 (0.801, 1.274) 0.929	0.989 (0.836, 1.171) 0.901	0.592 (0.355, 0.987) 0.044	1.123 (0.743, 1.696) 0.582	0.842 (0.612, 1.159) 0.292
B	0.893 (0.753, 1.060) 0.197	0.819 (0.648, 1.035) 0.094	0.917 (0.777, 1.081) 0.299	0.850 (0.545, 1.325) 0.472	0.871 (0.571, 1.328) 0.521	0.764 (0.558, 1.047) 0.094
AB	1.000 (0.790, 1.265) 0.998	0.876 (0.631, 1.216) 0.429	1.037 (0.828, 1.299) 0.753	0.718 (0.365, 1.412) 0.337	1.903 (1.190, 3.042) 0.007	0.773 (0.491, 1.216) 0.265
RhD (-)	1.000	1.000	1.000	1.000	1.000	1.000
RhD (+)	1.548 (0.523, 4.583) 0.430	1.471 (0.340, 6.359) 0.605	1.751 (0.592, 5.184) 0.312	0.203 (0.059, 0.698) 0.011	N/A	N/A

Significant difference was underlined font. N/A, not applicable (too low number of events).

Table 6. The association of ABO and RhD blood types with different thyroid abnormalities adjusted for maternal age.

	TPOAb(+) (n = 1,155)	TGAb(+) (n = 549)	TPOAb(+) or TGAb(+) (n = 1,306)	TRAb(+) (n = 116)	Clinical or subclinical hypothyroidism (n = 172)	Clinical or subclinical hyperthyroidism (n = 270)
	aOR (95% CI) p	aOR (95% CI) p	aOR (95% CI) p	aOR (95% CI) p	aOR (95% CI) p	aOR (95% CI) p
O	1.000	1.000	1.000	1.000	1.000	1.000
A	0.931 (0.780, 1.111) 0.427	1.011 (0.802, 1.276) 0.924	0.992 (0.838, 1.174) 0.923	0.593 (0.355, 0.990) 0.046	1.120 (0.741, 1.693) 0.589	0.841 (0.611, 1.157) 0.287
B	0.893 (0.752, 1.060) 0.197	0.819 (0.648, 1.035) 0.094	0.917 (0.777, 1.081) 0.302	0.847 (0.542, 1.322) 0.464	0.872 (0.571, 1.330) 0.523	0.763 (0.557, 1.045) 0.092
AB	0.999 (0.789, 1.265) 0.993	0.871 (0.627, 1.210) 0.411	1.036 (0.827, 1.299) 0.759	0.708 (0.359, 1.396) 0.319	1.902 (1.189, 3.042) 0.007	0.766 (0.487, 1.206) 0.250
RhD (-)	1.000	1.000	1.000	1.000	1.000	1.000
RhD (+)	1.586 (0.535, 4.703) 0.406	1.494 (0.345, 6.466) 0.591	1.796 (0.605, 5.325) 0.291	0.201 (0.058, 0.701) 0.012	N/A	N/A

Significant difference was in bold font. N/A, not applicable (too low number of events).

en, which can lead to adverse obstetric outcomes and have deleterious effects on both maternal health and fetal development. However, up to now, the relationship of ABO and RhD blood types with thyroid abnormalities in pregnant women has not been reported yet. In the present study, we found that pregnant women with blood type A and B had significantly higher concentrations of TSH than women with blood type O, and FERR

and B12 levels were remarkably increased in RhD(+) women than in RhD(-) women. Furthermore, logistic regression analysis revealed that pregnant women with blood type A were less likely to be TRAb(+), and pregnant women with blood type AB were more likely to suffer from clinical or subclinical hypothyroidism, compared with blood type O. In addition, RhD(+) pregnant women had less likelihood to be TRAb(+) in comparison to RhD(-) women.

Thyroid autoimmunity, as one of the most common autoimmune disorders, refers to the presence of autoantibodies including TPOAb, TGAb, or TRAb. TRAb plays an important and direct role in the pathogenesis of Graves' disease, while the roles of TPOAb and TGAb remain unclear. Thyroid autoantibodies can freely cross the placenta. TRAb might be responsible for fetal and neonatal hyperthyroidism. TPOAb and TGAb are important markers of thyroid autoimmunity, but they are not involved in fetal thyroid function [4]. Several risk factors have been reported for thyroid autoimmunity, such as older age, a family history of AITD, excess or deficient iodine intake, and European ancestry. The incidences of TPOAb and TGAb positivity in women of childbearing age are reported to increase with age. A national survey of the USA from 1988 to 1994 revealed that the prevalences of TPOAb and TGAb positivity were 9.2% and 11.3% in women aged 20 - 29 years old, 14.5% and 14.2% in women aged 30 - 39, and 16.4% and 18.0% in women aged 40 - 49, respectively [15]. Studies from Denmark and Sweden showed that the incidence of Graves' disease increased with age in women [16,17]. In our study, we also found that older age in pregnant women was significantly associated with the likelihood of thyroid autoimmunity. The older maternal age was, the higher the risk of getting TPOAb(+), TGAb(+), TPOAb(+) or TGAb(+), and TRAb(+) became, which was consistent with the findings of previous studies.

The blood group system has long been investigated to be associated with diverse diseases. Dahlen T et al. agnostically explored the association of ABO and RhD blood types with disease occurrence for a large number of disease phenotypes using large-scale Swedish healthcare registries, and found 49 and 1 associations between a disease and ABO and RhD blood system, respectively. Thyrotoxicosis was shown to be less frequent in blood type A and AB compared with blood type O. Their findings supply further support for previously known associations and also indicate new meaningful relations [11]. Afterwards, another study involving a Danish cohort of 482,914 patients using up to 41-years of follow-up data revealed that ABO and RhD blood groups were correlated with a wide spectrum of diseases including cancers, cardiovascular, endocrinal, infectious, and gastrointestinal diseases, etc. Thyrotoxicosis with or without goiter was found to be more common in blood group O as compared to non-O [10]. In our study, we found significant associations between the blood group system and thyroid abnormalities in pregnant women. Furthermore, after adjustment for maternal age, the relationships persisted. TRAb(+) was less likely to be observed in blood type A, and clinical or subclinical hypothyroidism was more frequently observed in blood type AB, compared with blood type O. In addition, TRAb(+) was found to be less common in RhD(+) than in RhD(-) women. Our findings indicate novel meaningful correlations between ABO and RhD blood types and thyroid disorders in pregnant women, which will offer guidance for better screening and management of thyroid function in pregnant women.

In addition, our study showed that concentrations of serum TSH were significantly higher in pregnant women with blood type A and B than in blood type O, which further suggested that the ABO blood group system was associated with thyroid function in pregnant women, though the biological significance of the TSH level difference remained unknown. Blood group antigens are found not only on red blood cells, but also on leukocytes, platelets, certain tissues, plasma proteins, and various cell surface enzymes. And they also exist in soluble form in body secretions such as breast milk, saliva, sweat, urine, seminal fluid, gastric secretions, and amniotic fluid [18]. Blood group antigens have wide functional diversity, and can be classified into five categories including transporters and channels, receptors for exogenous ligands, bacteria, viruses, and parasites, adhesion molecules, structural proteins, and enzymes [19]. ABO blood group antigens may influence host susceptibility to various diseases by different known or unknown mechanisms. Of great significance is the finding that ABO blood group and secretor status are significantly associated with the composition of human intestinal microbiome [20,21]. Gut microbiome plays an important role in mammalian homeostasis, such as maintaining gastrointestinal health, providing essential nutrients, assuring appropriate development of the immune system and catabolizing dietary fiber into short chain fatty acids [22]. Compelling evidence reveals that gastrointestinal health is essential for thyroid function directly and absorption of thyroid-associated nutrients. Gastrointestinal conditions are related with autoimmunity, and especially with thyroid autoimmunity [23]. So, the differences of ABO-blood-type-associated gut microbiome may affect host susceptibility to thyroid disorders. Of course, this is just a preliminary inference, and the exact mechanisms remain to be further investigated.

Importantly, in this study we also found that FERR and B12 levels were remarkably increased in RhD(+) than in RhD(-) women. Iron and vitamin B12 are two important nutrients required for thyroid health. It has been proposed that iron deficiency can adversely affect thyroid function of reproductive-age and pregnant women, leading to higher risk of TPOAb(+) and both TPOAb(+) and TGAb(+), increased TSH level, decreased FT4 level, and increased prevalence of overt and subclinical hypothyroidism [24]. Studies showed that B12 level was significantly lower in patients with AITD than in controls and was inversely correlated to TPOAb level [25,

26]. However, we didn't find any association between RhD blood group and TPOAb(+) or TGAb(+). Conversely, the association between RhD blood group and TRAb(+) was shown. So, the difference of FERR and B12 levels between RhD(+) and RhD(-) women might play a role in thyroid function, although its exact biological meaning needs to be clarified. Disease risk is obviously multifactorial and blood group antigens may be one of the predisposing factors that contribute to or prevent disease progression.

Our study has some limitations. First, it is a single-center, cross-sectional study. It should be prudent to extrapolate our results to other regions. The number of RhD(-) subjects is relatively low, which might affect the power of test. Second, we only included maternal age as a confounding factor. However, other factors such as ethnicity, iodine intake, and medical history of other autoimmune diseases might also be involved. More than 99% permanent population in Qingdao district belong to Han nationality. So, ethnicity won't affect the results of our study. Iodine intake in Qingdao district is generally sufficient, since Qingdao is a coastal city with rich seafood supplies. In addition, we detected urinary iodine concentration (UIC) for nearly 500 pregnant women during their first-trimester gestation and found that 89.66% cases had UIC between 150 - 499 μg/L (data not shown). Information about medical history of other autoimmune diseases was collected from 2,200 subjects, and the occurrence rates among different blood types showed no difference. So, despite the limitations inherent in our research, we believe that our findings are reliable and significant.

Ethical Approval:

The study followed the principles of the Declaration of Helsinki, and was approved by the ethics committee of Qingdao Women and Children's Hospital Affiliated to Qingdao University (approval number: QFELL-KY-2023-03).

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Declaration of Interest:

The authors declare that there are no conflicts of interest to disclose.

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