Effects of levothyroxine treatment on pregnancy outcomes in pregnant women with autoimmune thyroid disease

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Abstract

Background: Despite some studies indicating that thyroid antibody positivity during pregnancy has been associated with adverse pregnancy outcomes, evidence regarding the effects of levothyroxine (LT4) treatment of euthyroid/subclinical hypothyroid pregnant women with autoimmune thyroid disease on pregnancy outcome is limited. We aimed to assess whether pregnant women with autoimmune thyroid disease, but without overt thyroid dysfunction are affected by higher rates of adverse pregnancy outcomes. In addition, we aimed to explore whether LT4 treatment improves the pregnancy outcome of affected women.

Methods: A prospective study was carried out on pregnant women from the first trimester to delivery. The study was conducted among pregnant women receiving prenatal care in centers under coverage of Shahid Beheshti University of Medical Sciences. Of a total of 1746 pregnant women, screened for thyroid dysfunction, 1028 euthyroid TPOAb-negative (TPOAb⁻) and 131 thyroid peroxidase antibody-positive (TPOAb⁺) women without overt thyroid dysfunction entered the second phase of the study. TPOAb⁺ women were randomly divided into two groups: group A ($n=65$), treated with LT4 and group B ($n=66$), received no treatment. The 1028 TPOAb⁻ women (group C) served as a normal population control group. Primary outcomes were preterm delivery and miscarriage and secondary outcomes included placenta abruption, still birth, neonatal admission and neonatal TSH levels.

Results: Groups A and C displayed a lower rate of preterm deliveries compared with group B (RR=0.30, 95% CI: 0.1–0.85, $P=0.0229$) and (RR=0.23, 95% CI: 0.14–0.40, $P<0.001$) respectively. There was no statistically significant difference in the rates of preterm labor between groups A and C (RR=0.79, 95% CI: 0.30–2.09, $P=0.64$). The number needed to treat (NNT) for preterm birth was 5.9 (95% CI: 3.33–25.16).

Conclusions: Treatment with LT4 decreases the risk of preterm delivery in women who are positive for TPOAb.

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Introduction

Thyroid disorders, especially those of autoimmune origin, are common in women of reproductive age (1, 2). Although the complications of overt hypothyroidism or

hyperthyroidism on pregnancy outcomes, and neonatal and childhood development are well known (3), there is still no consensus on the association between subclinical

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thyroid disorders or autoimmune thyroid disorders and complications in pregnancy and childhood. Some studies have shown that subclinical hypothyroidism and thyroid autoimmunity are associated with a higher rate of placental abruption, preterm birth, miscarriage, gestational hypertension, fetal distress, severe preeclampsia, neonatal distress and gestational diabetes (4, 5, 6, 7), whereas others document controversial findings (8, 9, 10). A meta-analysis conducted by Sheehan *et al.* demonstrated no significant increase in odds ratio of preterm labor in women with subclinical hypothyroidism and isolated hypothyroxinemia (11), whereas Chan *et al.* reported a pooled odds ratio of 1.93 (1.40–2.64) for pregnancy loss, which included miscarriages, stillbirths and perinatal losses up till the first week of life (10).

A number of observational studies have reported that euthyroid TPO antibody-positive women are at increased risk of adverse outcomes of pregnancy such as miscarriage and premature labor or even neonatal complications (12, 13, 14); however, others found no association (9, 15, 16).

Despite the influence of thyroid autoimmunity reported in observational studies, even among euthyroid/subclinical hypothyroid pregnant women, there are limited trials on thyroxine supplements having beneficial effects on prenatal outcomes in this group (17, 18). As a result, various scientific societies, including the American Thyroid Association (ATA), European Thyroid Association (ETA) and the Thyroid Society report insufficient evidence and clinical trials on the effectiveness of levothyroxine treatments in these women in terms of pregnancy or neonatal outcomes. These societies mostly refer to a single clinical trial conducted by Negro *et al.* that reported a lower rate of adverse outcomes in TPO antibody-positive women treated with levothyroxine (18). In spite of this, in another study of infertile women undergoing assisted reproduction technologies, Negro *et al.* found LT4 treatment was not beneficial (19); neither did Lata *et al.* report any differences in the incidence of miscarriage among hypothyroid/euthyroid thyroid antibody-positive women (17). Generally, data available are lacking in recommendations for/against routine levothyroxine therapy during pregnancy in thyroid antibody-positive euthyroid/subclinical hypothyroid women.

The purpose of this population-based study was to identify whether TPOAb⁺ women without overt thyroid dysfunction are affected by higher rates of pregnancy complications and to determine the efficacy of levothyroxine on the outcomes of pregnancy in these women.

Subjects and methods

Study design and participants

This study was conducted in two phases, the first of which was a population-based cross sectional study in which 1746 pregnant women, attending prenatal clinics of Shahid Beheshti Medical University, were screened for thyroid dysfunction by collecting data on medical history, clinical examination and measurement of serum concentrations of TSH, T4 (TT4), T-uptake and TPOAb. By excluding those with twin pregnancies ($n=28$), those diagnosed with hyperthyroidism or overt hypothyroidism and those TPOAb⁻ subclinical hypothyroid women, 134 TPOAb⁺ (euthyroid and subclinical) women and 1092 euthyroid TPOAb⁻ women remained and were invited for the second phase of the study; of these, 1028 and 131 women accepted respectively (Fig. 1). The second phase of this study was a single blind clinical trial conducted on TPOAb⁺ subjects, divided into two groups, group A ($n=65$), treated with LT4 and group B ($n=66$), without treatment; TPOAb⁻ women with normal TSH and FT4 served as the control group (group C); all three groups were followed and adverse outcomes of pregnancy including preterm delivery, miscarriage, placenta abruption, still birth and neonatal admission. Secondary outcomes were placenta abruption, still birth, neonatal admission and neonatal TSH levels were documented. LT4 administration was initiated 4–8 days after the first prenatal visit in group A.

We used the same protocol for intervention as Negro *et al.* did (18); those in the intervention group received a morning dose of 0.5 µg/kg/day if they had TSH <1.0 µIU/mL, 0.75 µg/kg/day for TSH between 1.0 and 2.0 µIU/mL, and a 1 µg/kg/day dose for TSH >2.0 µIU/mL or a TPOAb titer exceeding 1500 IU/mL; dosages were maintained throughout gestation.

Written informed consent was obtained from all participants, and the study was approved by the ethics committee of the Research Institute of Endocrine Sciences (RIES) (approval no: 32ECRIES92/07/23). This study is registered in the Iranian Randomized Clinical Trials Registry (IRCT, code: IRCT2013100114849N1).

Randomization

Pregnant women, positive for thyroid peroxidase antibodies (TPOAb), with normal TSH and FT4 were randomly divided into two groups. Randomization was performed in blocks of four using a computer-generated list. Physicians, who participated in various phases of

Figure 1

Study flowchart.

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the study, were blinded to grouping of patients; only the health care provider, who did not participate in any phase of the study, was aware of the group the patient was in.

Masking to treatment allocation was not possible; only those in a position to determine pregnancy outcome of mother and neonate were blinded to treatment allocation.

Study procedure

A comprehensive questionnaire including information on demographic, reproductive, medical and prenatal history was filled out, during face-to-face interviews; a checklist including all potential risk factors, as recommended by the American Thyroid Association (20), was completed for all participants and physical examinations including thyroid, weight, height, systolic and diastolic pressure were conducted; overnight blood samples were collected and after centrifugation were sent to the Research Institute of Endocrine Sciences (RIES) of Shahid Beheshti University of Medical Sciences. Serum concentration of thyroxine (T4), T-uptake, thyrotropin (TSH) and thyroid peroxidase antibody (TPOAb) were measured to determine the thyroid status of participants. As free T4 (FT4) immunoassays may be influenced by pregnancy-related changes of serum thyroxine-binding globulin and albumin, in a method-specific manner, to assess FT4 status, we used Free Thyroxine Index (FT4I) (21) calculated using the formula: $FT4I = T4 \times T\text{-uptake} / 100$. Three casual morning urine samples (5–10 mL) for each participant were collected on every other day basis and kept frozen at -20°C until assayed at the end of the study; the median urinary iodine was calculated. Blood samples were collected during the second (20- to 24-weeks gestation) and third (30- to 34-weeks gestation) trimesters; samples were obtained during fasting (before ingestion of LT4 in group A); serum was separated and stored at -80°C till the end of the study for measurement of thyroid hormones. Serum concentrations of neonatal TSH were measured from heel blood samples, 3–5 days after delivery. Gestational age was calculated according to the first day of their last menstrual cycle (LMP) for women with regular cycles and/or ultrasonography for those with irregular cycles or those who could not precisely recall their LMP ($n=66$).

Women with baseline TSH levels of 0.1–2.5 $\mu\text{IU/mL}$, FT4I 1–4.5 and TPO $<50\text{IU/mL}$ were considered euthyroid TPO[−] and served as controls. Overt hyperthyroidism was defined as TSH levels $<0.1\mu\text{IU/mL}$ and FT4I >4.5 .

Overt hypothyroidism was defined as TSH $>10\mu\text{IU/mL}$ or TSH levels $>2.5\mu\text{IU/mL}$ and FT4I <1 . Subclinical hypothyroidism was defined as normal FT4I (1–4.5), despite elevated TSH (2.5–10 $\mu\text{IU/mL}$). Subclinical hyperthyroidism was defined as normal FT4I (1–4.5), despite reduced TSH (TSH <0.1). Individuals with TPOAb levels $>50\text{IU/mL}$ were considered TPO⁺.

Using radioimmunoassay (RIA) and immunoradiometric assay (IRMA), T4 and TSH were measured using commercial kits (Izotop Kit, Budapest co, Hungary) and the Gamma-counter (Dream Gamma-10, Goyang-si, Gyeonggi-do, South Korea); T-uptake and TPOAb were measured by the enzyme immunoassay (EIA) (Diaplus Kit, San Francisco, CA, USA) and immunoenzymometric assay (IEMA) (Monobind Kit, Costa Mesa, CA, USA) respectively using a calibrated ELISA reader (Sunrise, Tecan Co. Salzburg, Austria). Inter-assay and intra-assay coefficients of variation for T4, T-uptake, TSH and TPOAb were 1.1% and 3.9%, 2.2% and 4.3%, 1.9% and 4.7% and 1.0% and 1.6% respectively.

Urinary iodine concentration was measured using a manual method, based on the Sandell–Kolthoff technique (22). The intra-assay (coefficient variation) in three ranges of 3.4, 12.5 and 37.1 $\mu\text{g/L}$ were 8.5, 7.2 and 9.6% respectively, and inter-assay CVs % were 9.1, 8.6 and 12.3% respectively.

Outcomes

In this study, the primary outcome was preterm delivery, defined as birth before 37-weeks' gestation. Secondary outcomes were placenta abruption, still birth, neonatal admission and neonatal TSH levels. Miscarriage was defined as the loss of an embryo or fetus before the 20th week of pregnancy. Placental abruption was defined as separation of the placenta from the wall of the uterus during pregnancy. Still birth was defined as birth of an infant that died in the mother's uterus, after 20 weeks of gestation. Newborn admission was defined as the admission of a neonate to the neonatal unit, mainly due to fetal distress or icterus.

Statistical analysis

Sample size was calculated for intention for treatment analysis with superiority assumption (in terms of primary outcome). A sample of 130 (65 subjects per group) was needed to detect a reduction in preterm delivery of 10% in the intervention group, compared to 30% in the control

Table 1 Characteristics of women participants in the 2nd phase according to the study groups.

Characteristics	Group A (n=65)	Group B (n= 66)	P value	Group C (n= 1028)
Maternal age* (year), Mean (s.d.)	26.6 (5.82)	27.0 (4.67)	0.680	27.1 (5.17)
Maternal BMI* (kg/m ²), Mean (s.d.)	24.9 (5.12)	24.6 (3.59)	0.704	24.8 (4.61)
Gestational age at first visit* (week), Mean (s.d.)	10.78 (3.97)	11.58 (3.99)	0.248	11.20 (4.11)
Gestational age at first visit**, n (%)				
<8 weeks	24 (36.9)	20 (30.3)		328 (30.0)
8–10 weeks	8 (12.3)	6 (9.1)	0.864	228 (20.9)
10–12 weeks	11 (16.9)	12 (18.2)	0.601	164 (15.0)
12–14 weeks	10 (15.4)	14 (21.2)	0.310	111 (10.2)
14–20 weeks	12 (18.5)	14 (21.2)	0.497	261 (23.9)
Systolic blood pressure*** (mmHg)				
First visit	106.7	106.2	0.175	106.5
2nd trimester	101.2	103.6	0.574	102.6
3rd trimester	100.7	105.8	0.104	103.5
Diastolic blood pressure*** (mmHg)				
First visit	67.3	64.9	0.416	67.4
2nd trimester	66.8	65.7	0.954	64.8
3rd trimester	62.1	63.8	0.309	65.7
Reproductive history**, n (%)				
Primigravida	19 (29.6)	18 (27.3)	0.803	377 (34.5)
Multigravida	46 (70.7)	48 (72.7)		715 (65.5)
History of infertility, n (%)	0	0		48 (4.4)
TSH*** (μIU/mL)				
1st trimester	3.7 (2.4–4.8) ^α	3.2 (2.1–5.2) ^γ	0.907	1.5 (0.80–1.9)
2nd trimester	1.5 (0.99–2.0) ^α	3.9 (2.9–5.4) ^γ	<0.001	1.8 (1.2–2.4)
3rd trimester	0.97 (0.60–1.8) ^{α,†}	3.4 (2.0–5.2) ^γ	<0.001	1.7 (1.0–2.3)
Neonate	1.3 (0.45–1.9)	1.0 (0.43–1.9)	0.451	0.90 (0.40–1.7)
FT4I***				
1st trimester	2.7 (2.3–3.4)	2.8 (2.3–3.1) [‡]	0.870	3 (2.4–3.4)
2nd trimester	3.1 (2.7–3.7)	2.8 (2.2–3.0) [‡]	<0.005	3.1 (2.6–3.5)
3rd trimester	2.9 (2.5–3.1)	2.7 (2.2–3.0) [‡]	0.198	3 (2.6–3.2)
Urine iodine*** (μg/L), Median (IQ)	84.52 (58.51, 137.23)	104.78 (78.59, 140.24)	0.227	119.70 (78.72, 184.27)

Group A, TPOAb⁺ women treated with LT4; group B, TPOAb⁺ women with no treatment; group C, control group.*TSH in Group A in comparison to group B; $P < 0.001$; ^γTSH in group B in comparison to group C; $P < 0.001$; [‡]TSH in group A in comparison to group C; 1st and 3rd trimester $P < 0.001$, 2nd trimester $P < 0.05$; ^γFT4I in Group A in comparison to group B; 2nd trimester $P < 0.005$; [†]FT4I in group B in comparison to group C; 1st trimester $P < 0.05$, 2nd and 3rd trimester $P < 0.001$; * t -test was used to compare groups; ** χ^2 test was used to compare groups; ***Mann–Whitney test was used to compare the groups and data for TSH and FT4I is presented as median (percentiles 25–75).

group, with a two-sided 5% significance level, a power of 80% and a loss to follow-up of 10%. The calculated sample size for the first phase of the study was 1600, among these, 122 euthyroid/subclinical TPOAb⁺ women were detected who did not provide us enough cases in each study group; hence, recruitment of pregnant women was continued until we had 1746 subjects, which resulted in 131 women with TPOAb⁺.

All analyses were by intention to treat. No participants were excluded from the primary intention-to-treat analysis for protocol violations. The primary outcome was calculated as event numbers and percentages by treatment allocation. Effect measures (relative risks (RRs)) were calculated with 95% CIs, with expectant management as the reference group. There was no imputation for missing outcomes. Participants with missing data were excluded from calculation. Number needed to treat (NNT) was defined as the numeric cohort of patients who need to be treated to prevent the occurrence of primary outcome.

Continuous variables were checked for normality using the one-sample Kolmogorov–Smirnov test; categorical variables are expressed as percentages and were compared using Pearson's χ^2 test. Continuous variables with normal distribution were compared between the two groups, using *t*-test and are expressed as mean $\pm$ s.d. Non-normal distributed variables, expressed as median (interquartiles), were compared, using Mann–Whitney test. The one-way analysis of variance (ANOVA) was used to determine whether there were any differences between the means of gestational age, birth weight, birth height and birth head circumference in the three study groups. An ANOVA with repeated measure was used to compare means of the three groups, for which participants were measured three times to see if there were any changes in TSH and FTI. The effect

of treatment on mediated variable (preterm delivery) was estimated using mediation analysis (23). Statistical analysis was performed using SPSS software, version 18.

Results

Mean $\pm$ s.d.s of age and gestational age of the women were 26.7 ± 5.14 years and 11.4 ± 4.2 weeks respectively; 1204 (75.3%) women were <14 weeks pregnant and 396 (24.7%) were between 14 and 20 weeks. Mean $\pm$ s.d. of BMI was $25.3 (\pm 7.47)$ kg/m². Of pregnant women, 636 (36.4%) were primigravida and 1110 (63.6%) multigravida; there was history of infertility in 96 (5.5%) pregnant women.

Using the predefined classification, after excluding twins ($n=28$), 36.4% (626) had thyroid disorders, including 0.8% ($n=14$) overt hyperthyroidism, 3.5% ($n=60$) overt hypothyroidism, 1.5% ($n=25$) subclinical hyperthyroidism, 22.9% ($n=393$) TPOAb[−] subclinical hypothyroidism, 7.8% ($n=134$) euthyroid/subclinical hypothyroidism TPOAb⁺ and 63.6% ($n=1092$) were euthyroid TPOAb[−]; the last two groups (1092 and 134 women) were invited for the 2nd phase of the study, of which 1028 and 131 accepted respectively. Eventually 940 and 114 women completed the study (Fig. 1); there were no significant differences in terms of age, education, parity and gestational age at the initiation of the study between those who completed the study and those who did not.

Characteristics of women who participated in phase 2, based on the study groups (A, B and C) are presented in Table 1. There were no statistically significant differences between demographics, anthropometrics and the reproductive history of study groups; nor were there any significant differences in mean birth weight, head circumference (Table 2) and neonate TSH levels

Table 2 Pregnancy outcomes in study groups.

Pregnancy adverse outcomes	Group A ($n=56$)	Group B ($n=58$)	Group C ($n=940$)
Miscarriage*, n (%)	2 (3.6)	2 (3.4)	40 (4.3)
Placental abruption*, n (%)	0	0	5 (0.5)
Preterm delivery*, n (%)	4(7.1) [†]	14(23.7) [‡]	53 (5.6)
Still birth*, n (%)	0	0	2 (0.2)
Neonatal admission*, n (%)	2 (3.6) [†]	12 (20.7) [‡]	75 (8.0)
Gestational age**, mean (s.d.)	39.3 (1.3)	38.4 (± 1.7)	39.4 (± 1.4)
Birth weight**, mean (s.d.)	3139.1 (± 287.6)	3127.7 (± 523.5)	3236.6 (± 448.8)
Birth height**, mean (s.d.)	49.5 (± 1.7)	50.3 (± 1.5)	50.1 (± 2.3)
Birth head circumference**, mean (s.d.)	34.5 (± 1.1)	34.9 (± 1.4)	34.7 (± 1.6)

Group A, TPOAb⁺ women treated with LT4; group B, TPOAb⁺ women no treatment; group C, control group.

[†]Group A vs group B; $P < 0.05$; [‡]group B vs group C; $P < 0.005$; ^{*} χ^2 test was used to compare groups; ^{**}ANOVA test was used to compare groups.

Miscarriage, loss of an embryo or fetus before the 20th week of pregnancy; Placental abruption, separation of the placenta from the wall of the uterine during pregnancy; Preterm delivery, birth before 37 weeks gestation; Still birth, birth of an infant that died in the mother uterine after 20 weeks of gestation; Newborn admission, admission of neonate to neonatal unit mainly due to fetal distress or icterus.

between the study groups (Table 1). Neither was there any statistically significant correlation of neonatal TSH with first, second and third trimester maternal TSH values, in any of the study groups.

The medians (interquartiles) of urine iodine in groups A, B and C were 84.52 (58.51, 137.23), 104.78 (78.59, 140.24) and 119.70 (78.72, 184, 27) µg/L respectively, indicating no significant difference between these groups (Table 1).

Figure 2 shows FT4I (2-A) and TSH values (2-B) of study groups throughout the entire gestation period (1st, 2nd and 3rd measurement); medians (interquartiles) FT4I in group A in the 1st, 2nd and 3rd trimesters were 2.7 (2.3–3.4), 3.1 (2.7–3.7) and 2.9 (2.5–3.1) respectively. Although the first TSH value in group A was not significantly different from that in group B ($P=0.841$, median (interquartiles): 3.7 (2.4–4.8) and 3.2 (2.1–5.2) µIU/mL respectively), in the 2nd and 3rd trimesters, it was significantly higher in group B, compared to group A ($P<0.001$, 3.9 (2.9–5.4) vs

Table 3 TSH levels in study groups during pregnancy.

TSH levels	Group A	Group B	Group C
TSH, 1st visit (µIU/mL) (%)			
<2.5	30.8	40.9	100.0
2.5–5	49.2	28.8	0.0
5–10	20.0	30.3	0.0
TSH, 2nd trimester (µIU/mL) (%)			
<2.5	81.5	19.7	76.4
2.5–5	18.5	40.9	23.6
5–10	0.0	39.4	0
TSH, 3rd trimester (µIU/mL) (%)			
<2.5	87.7	25.8	85.9
2.5–5	10.8	36.4	14.1
5–10	0.0	37.9	0.0

Group A, TPOAb⁺ women treated with LT4; group B, TPOAb⁺ women with no treatment; group C, control group.

1.5 (0.99–2.0) and 3.4 (2.0–5.2) vs 0.97 (0.60–1.8) µIU/mL respectively) (Table 1). Table 3 shows the percentages of women with TSH values <2.5, 2.5–5 and 5–10 µIU/mL in each trimester according to the study groups. Although none of the participants in group A had TSH values between 5 and 10 µIU/mL in the 2nd and 3rd trimesters, 39.4% and 37.9% of women in group B had TSH values within this range in these trimesters respectively (Table 3).

The rates of preterm deliveries in groups A and C were lower than those of group B (RR=0.30, 95% CI: 0.1–0.85, $P=0.0229$) and (RR=0.23, 95% CI: 0.14–0.40, $P<0.001$) respectively. There was no significant difference in the rates of preterm labor between groups A and C (RR=0.79,

Figure 2

FT4I (2A) and TSH (2B) values during pregnancy.

Figure 3

Preterm delivery and neonatal admission among study groups. A full colour version of this figure is available at <http://dx.doi.org/10.1530/EJE-16-0548>.

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95% CI: 0.30–2.09, $P=0.64$) (Fig. 3 and Table 2). The number needed to treat (NNT) for the preterm birth was 5.9 (95% CI: 3.33–25.16).

The rates of neonatal admission in groups A and C were significantly lower than those in group B ($P=0.005$, RR=0.17, 95% CI: 0.04–0.73) and ($P=0.001$, RR=0.38, 95% CI: 0.22–0.66) respectively) (Fig. 3 and Table 2). The number needed to treat (NNT) for neonatal admission was 5.84 (95% CI: 3.48–18.23). In addition, by adjusting the effect of intervention on NICU for preterm status, the risk of neonatal admission in term neonates of the ‘no LT4’ group was twice that of the LT4 group ($P=0.3$, RR=0.42, 95% CI: 0.072–2.38), although it was not significant as the majority of admissions to NICU were preterm. Mediation analysis demonstrated that 50% of the decrease in the risk of neonatal admission due to treatment was mediated via the decrease in premature delivery after LT4 treatment ($RR_{AC}/RR_{AB}=\log(0.42)/\log(0.17)=0.5$).

There were no significant differences in mean birth weight, head circumference and neonate TSH levels between the study groups (Tables 1 and 2); neither were there any significant correlations between the first, second and third trimester maternal TSH values with neonatal TSH, in any of the study groups. Two cases in group A stopped taking LT4 and none of group B or C took LT4, excluding these two cases did not change the results significantly.

Subgroup analysis of data, based on cut-off values of 4 μ IU/mL for TSH and 150 μ g/L for urinary iodine is presented in Tables 4 and 5; using these cut-off values demonstrated no significant differences in terms of preterm delivery or neonatal admission between those women in groups A and B with TSH level of <4 μ IU/mL, but there were significant differences in preterm delivery and neonatal admission of women in groups A and B, who had baseline TSH values of ≥ 4 μ IU/mL (5.3% vs 29.4%; $P=0.01$) and (0 vs 29.4%, $P<0.001$) respectively. Preterm delivery was observed in 13 (28.2%) and 1 (8.3%) women in group B with urinary iodine <150 μ g/L and ≥ 150 μ g/L

respectively, a difference not statistically significant ($P=0.26$).

Discussion

This study revealed that administration of levothyroxine in TPOAb⁺ pregnant women with no overt thyroid dysfunction can lead to 70% and 83% decrease in preterm delivery and neonatal hospital admissions respectively. We found that by treating 5.9 TPOAb⁺ pregnant women with levothyroxine, one preterm delivery could be prevented. The beneficiary effect of LT4 treatment was mainly observed among TPOAb⁺ women with TSH ≥ 4 μ IU/mL; in women receiving LT4, preterm delivery was 5.3%, whereas it was 29.4% among those without the treatment ($P=0.01$).

It is well known that overt thyroid dysfunction is associated with pregnancy complications (3, 11). One meta-analysis demonstrated a 1.19- and 1.24-fold increase in risk of preterm delivery in pregnant women with overt hypothyroidism and hyperthyroidism, compared to the reference group respectively (11). However, the association between TPOAb⁺ in euthyroid/subclinical pregnant women and adverse pregnancy outcomes is not yet fully understood, and the underlying mechanisms involved have not been clarified (24). Thyroid autoantibodies may exert their adverse effects on pregnancy outcomes in both a TSH-dependent and a TSH-independent manner. The alteration in TSH/T4 levels has a clear antibody-dependent component; it has been shown that baseline TSH levels of pregnant women were significantly higher in TPO⁺ women than in TPO negative women, whereas they were well within the euthyroid range (25). It seems that women who are positive for thyroid antibodies before pregnancy may have subtle preexisting thyroid dysfunction that could possibly worsen during pregnancy (26, 27). Besides these women may not be able to adequately respond to their demand for the augmented synthesis of thyroid

Table 4 Pregnancy outcomes in study groups based on cut-off values of 4 μ IU/mL for TSH.

	TSH value						
	<4 μIU/mL			≥4 μIU/mL			
	Group A (n=18)	Group B (n=24)	P value	Group A (n=38)	Group B (n=34)	P value	Controls
Adverse pregnancy outcomes							
Preterm delivery, n (%)	2 (11.1)	4 (16.7)	0.69	2 (5.3)	10 (29.4)	0.01	– – –
Neonatal admission, n (%)	2 (11.1)	2 (8.3)	0.99	0 (0)	10 (29.4)	<0.001*	– – –

Group A, TPOAb⁺ women treated with LT4; group B, TPOAb⁺ women with no treatment; group C, control group. χ^2 test was used to compare groups.

*Fisher's exact test was used to compare groups.

Preterm delivery, birth before 37 weeks' gestation; Neonatal admission, admission of neonate to neonatal unit mainly due to fetal distress or icterus.

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Table 5 Pregnancy outcomes in study groups based on cut-off values of 150 µg/L for urinary iodine.

Adverse pregnancy outcomes	Urinary iodine								
	Group A			Group B			Controls		
	<150 µg/L (n=37)	≥150 µg/L (n=19)	P value*	<150 µg/L (n=46)	≥150 µg/L (n=12)	P value*	<150 µg/L (n=540)	≥150 µg/L (n=400)	P value*
Preterm delivery, n (%)	3 (8.1)	1 (5.3)	0.99	13 (28.2)	1 (8.3)	0.26	28 (5.2)	25 (6.3)	0.48
Neonatal admission, n (%)	2 (5.4)	0 (0)	0.54	9 (19.6)	3 (25.0)	0.70	48 (8.9)	27 (6.8)	0.23

Group A, TPOAb⁺ women treated with LT4; group B, TPOAb⁺ women with no treatment; group C, control group. χ^2 test was used to compare groups.

*Fisher's exact test was used to compare groups.

Preterm delivery, birth before 37 weeks' gestation; Neonatal admission, admission of neonate to neonatal unit mainly due to fetal distress or icterus.

hormones required during pregnancy (28); as a result subclinical or overt hypothyroidism may occur during pregnancy (26, 27, 28). Thyroid autoantibodies may exert their effect in a TSH-independent manner that includes quantitative and qualitative changes in the profile of endometrial T cells with reduced secretion of IL-4 and IL-10 along with hypersecretion of interferon γ (29). Besides the hyperactivity and increased migration of cytotoxic natural killer cells that alter the immune and hormonal response of the uterus are up to 40% more common in women with thyroid autoimmunity (29). It has also been shown that thyroid autoimmunity may represent a marker of a generalized autoimmune imbalance that could result in dysregulated activity of the immune system at the fetal-maternal interface (14, 30).

Despite the known association between overt thyroid dysfunction and preterm labor, data on the relationship between subclinical thyroid dysfunction and thyroid autoimmunity with preterm labor are inconsistent. Although one meta-analysis, based on fourteen cohort studies and one case control study, demonstrated no significant increase in odds ratio of preterm labor in women with subclinical hypothyroidism (11), another reported a pooled odds ratio of 1.30 (1.05–1.60) for preterm delivery in subclinical hypothyroidism, compared to women with euthyroidism, using a random effects model (10). It is assumed that thyroid autoimmunity may be associated with an increased risk of preterm labor due to an inadequate response to the additional demand for thyroid hormones required during pregnancy (28) or because of dysregulation of local inflammatory processes involved in the cytokine networks of the local placental-decidual environment (31, 32, 33, 34, 35), dysregulation, which *per se* can have a direct adverse effect on placental or fetal development (18, 28). Besides lack of vitamin D has been suggested as a predisposing factor to autoimmune diseases and is known to be reduced in patients with thyroid autoimmunity (36); in turn, its deficiency is also linked

to pregnancy loss, suggesting a potential interplay with thyroid autoimmunity in the context of adverse pregnancy outcomes (37). Some studies, in agreement with ours, have reported a 2- to 3-fold increase in preterm delivery in euthyroid/subclinical TPOAb⁺ pregnant women (6, 8, 38, 39, 40), whereas others did not report this association (15, 41). A meta-analysis by Van den Boogaard *et al.* also showed that the presence of thyroid antibodies has been associated with a 1.9-fold increase in risk of premature birth (42); these differences in results might be due to differences in study design, age differences in study groups, BMI, ethnicities, iodine status and so forth. For instance, in some studies, women with antibodies were older than those without the antibodies; hence, older age, *per se*, may explain the increased rate of fetal loss (43). Studies on the effectiveness of levothyroxine treatment on adverse fetomaternal outcomes have documented inconsistent results. Negro *et al.*, in agreement with results of the current study, found that TPOAb⁺ pregnant women (euthyroid and subclinical hypothyroidism) are associated with an increased risk of preterm delivery, and treatment with levothyroxine can reduce the risk (18); Lata *et al.*, however, reported no effect of LT4 treatment on miscarriage among women with hypothyroidism and euthyroid TPOAb⁺ women (17).

Thyroid autoantibodies are believed to increase early pregnancy loss through changes in the profile of endometrial T cells, hyperactivity and increased migration of cytotoxic natural killer cells, affecting zona pellucida, human chorionic gonadotropin receptors and other placental antigens (44, 45, 46). Nevertheless, data regarding the miscarriage consequence of thyroid autoimmunity are inconsistent; despite some studies reporting a positive association between thyroid immunity and rate of miscarriage (7, 47, 48, 49, 50, 51, 52, 53, 54); others found no association between the presence of TPO antibodies and miscarriage (9, 55, 56, 57). We observed neither increase in rate of miscarriage among TPOAb⁺

Clinical Study	S Nazarpour and others	Levothyroxine treatment in TPOAb ⁺ pregnant women	176:2	262
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women nor a positive effect of LT4 treatment on reducing the rates of miscarriages; however, this is mainly due to the lack of LT4 administration in the very early stages of pregnancy, as the majority of miscarriages occur before 8 weeks of gestation (64.3% of women in our study had not started LT4 by the 8th week of gestation). Timing of treatment initiation is critical as it seems that normal thyroid function is primarily important in early pregnancy to maintain normal placental development and avoid miscarriages (18). Besides differences in gestational age of participants, age, BMI, ethnicity and iodine status of various populations also differ, which may partly explain the various associations between thyroid immunity and miscarriage. The present study was conducted in Iran, an iodine sufficient area according to the national surveillance study among Iranian students (58); however, we found that iodine sufficiency among students could not guarantee iodine sufficiency in pregnant women, and although the median level of urinary iodine was $>100\mu\text{g/L}$, it was still much lower than the level recommended for pregnant women ($150\mu\text{g/L}$).

Subgroup analysis of our data set, stratifying the analyses according to TSH value of $4\mu\text{IU/mL}$, showed that the beneficial effect of LT4 treatment on our primary outcomes was predominantly present in women with TSH $>4\mu\text{IU/mL}$. It seems that the TSH cut-off value of $2.5\mu\text{IU/mL}$ suggested by the American Thyroid Association (ATA) and European Thyroid Association (ETA) need to be revised, as it may result in an over diagnosis of subclinical hypothyroidism among pregnant women; the reference values for TSH in various populations, reported to be much higher than $2.5\mu\text{IU/mL}$, range from 2.63 to $4.68\mu\text{IU/mL}$ (59). In our study, apparently even using a cut-off value of $4\mu\text{IU/mL}$, LT4 treatment did not improve pregnancy outcomes in euthyroid TPOAb⁺ women, a finding similar to that of a recent RCT documented by Negro *et al.*; in which, however, they used a threshold of $2.5\mu\text{IU/mL}$ for identification of euthyroid women (60).

Regarding strengths and limitations of the present study, this is the second population-based randomized clinical trial, demonstrating the benefits of LT4 administration on adverse pregnancy outcomes in terms of preterm delivery; in addition, it showed decrease in neonatal admissions and improved maternal thyroid status throughout gestation; it is noteworthy that the iodine status of participants in this investigation has been assessed for the first time simultaneously with their thyroid hormonal assessment. The results of this study should be extrapolated considering the following

limitations: First, despite efforts for recruitment of pregnant women in the very early stages of pregnancy for administration of LT4 to those qualified for treatment, untimely referral of some women led to the initiation of treatment at the end of the first trimester, which could interrupt the precise assessment of LT4 effects on miscarriage; as a result, this negative association should be interpreted with caution as the study is underpowered to consider miscarriage as an outcome. Second, we did not use our local trimester-specific cut-off values for TSH and FT4I (21), as these values were introduced after the initiation of the present study. Third, sample size of this study did not allow subgroup analysis using TSH cut-off value of $2.5\mu\text{IU/mL}$ for comparison for euthyroid and subclinical hypothyroid TPOAb⁺ subgroups. Fourth, the number of samples was inadequate to examine other rare pregnancy complications, e.g. preeclampsia, stillbirth, etc. Fifth, lack of awareness regarding some other risk factors of miscarriage and premature delivery could influence the results of this study, although the randomized allocation of study participants minimizes this effect. Sixth, as the casual urine iodine values did not reflect iodine status of an individual (due to major day-to-day variability of urinary iodine excretion (61)), the subgroup analysis according to the urinary iodine's concentration needed to be interpreted with caution.

Conclusions

The results of the study showed that replacement therapy with levothyroxine in TPOAb⁺ pregnant women with normal FT4 improves pregnancy outcomes and is beneficial in reducing preterm delivery and neonatal admissions, a beneficial effect, however, mainly present in women with TSH $>4\mu\text{IU/mL}$. Larger randomized clinical trials conducted in various geographic regions with sufficient sample sizes and different iodine and TSH statuses are critically needed to investigate whether LT4 treatment benefits in TPOAb-positive women are dependent on TSH concentrations at initiation.

Declaration of interest

The 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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Author contribution statement

S N contributed to study design, execution, analysis, manuscript drafting and critical discussion. F R T contributed to study design, analysis, manuscript drafting and critical discussion. M S contributed to study design and manuscript drafting. M T contributed to laboratory testing and manuscript drafting. H A contributed to study design and analysis. F A contributed to study design, execution and manuscript drafting.

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