Reference Values for TSH and Free Thyroid Hormones in Healthy Pregnant Women in Poland: A Prospective, Multicenter Study

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Keywords

Pregnancy · Thyroid function · Thyroid tests · Reference values

Abstract

Objectives: The diagnosis and treatment of thyroid diseases in pregnant women remains a challenge. Various medical associations recommend establishing the reference intervals for thyroid hormones by a local laboratory. Considering differences within geophysical, socioeconomic conditions, and iodine prophylaxis in various populations, it is advisable to assess reference intervals for thyroid hormones specific to a region of residence. The objective was to assess trimester-specific reference intervals for TSH, fT₃, and fT₄ for pregnant women in the Polish population. **Methods and Re-**

sults: We conducted a prospective study in 4 centers representing different regions of Poland (Krakow, Warsaw, Poznan, and Białystok). Our study included consecutive, healthy pregnant women (172 patients), with an age range of 27–47 years. All women had a negative history for thyroid diseases, normal thyroid peroxidase antibody levels, and proper iodine prophylaxis. All newborns had TSH levels in the appropriate reference range. Serum TSH, fT₃, fT₄, and thyroid-peroxidase antibodies were measured in each trimester. The reference intervals were calculated using the percentile method, as recommended by the International Federation of Clinical Chemistry. The reference values calculated were 0.009–3.177, 0.05–3.442, and 0.11–3.53 mIU/L for TSH; 3.63–6.55, 3.29–5.45, and 3.1–5.37 pmol/L for fT₃; and 11.99–21.89, 10.46–16.67, and 8.96–17.23 pmol/L for fT₄ in consecutive trimesters of pregnancy. Reference intervals for

pregnant women when compared to the general population showed a lower concentration of TSH in every trimester of pregnancy and lower fT_4 in the 2nd and 3rd trimesters.

Conclusions: Using appropriate trimester-specific reference intervals may improve care of pregnant women by preventing misdiagnosis and inadequate treatment.

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Introduction

The diagnosis and treatment of thyroid diseases in pregnant women is a difficult and real challenge for the treating physician. Thyroid disorders in women of child-bearing age are frequent – they affect around 1.2% of pregnancies [1]. If left untreated, they cause obstetric complications and disorders in fetal development, including placental abruption, preeclampsia, intrauterine growth restriction, preterm birth, and fetal death. This applies to both overt and subclinical hypothyroidism and overt hyperthyroidism [2–7]. In addition, the fetuses of mothers with autoimmune thyroid disease are exposed to maternal antibodies, which may be a cause of neonatal thyroid disease [8, 9].

Thyroid function in pregnant women is modified by additional factors related to pregnancy: plasma volume increased up to 50%, increased levels of proteins binding thyroid hormones, including thyroid-binding globulin, human chorionic gonadotropin (hCG), an increase in the concentration of estrogens, and a relative iodine deficiency [10]. Accordingly, it can be expected that thyrotropin (TSH) and free thyroid hormones concentrations in normal pregnancy differ from those in the general population. Moreover, dynamic changes occur throughout pregnancy.

hCG is produced from the 8th day of pregnancy, initially by the embryo, then consequently by the syncytiotrophoblast. Due to the structural similarity to TSH, it acts as a stimulating agent for the thyroid. The hCG hormone has weak thyrotropic activity (approx. one 10th the potency of TSH) through the activation of the TSH receptor. Chorionic gonadotropin action reaches its peak at the end of the first trimester (max. at 9th to 11th week of gestation), and then gradually decreases. hCG secretion peak corresponds to the decrease in TSH secretion [11].

TSH levels during late pregnancy stay within the reference intervals determined for the general population, but in the first trimester it tends to be lower. The lowest TSH concentrations are found in the 10th to 12th week and then usually normalize in approximately the 18th week of

pregnancy. Multiple pregnancy enhances hCG increase and TSH decrease; hCG secretion peak is larger and longer [12].

On the other hand, concentrations of free thyroxine (fT_4) decrease in subsequent trimesters of pregnancy. This is a result of the thyroid response to the increasing level of thyroid-binding globulin, increased demands of the fetus for thyroid hormones in the first trimester of pregnancy, and the altered metabolism of thyroid hormones in the developing placenta in late pregnancy [10].

The Endocrine Society guidelines, published in 2012, regarding the treatment of thyroid disease during pregnancy and postpartum, recommend to determine reference intervals for thyroid hormones by local laboratories [13]. The reference ranges established for the general population are not relevant for pregnant women. Also, since the rapid hormonal changes during pregnancy must be addressed, there is a need for establishing trimester-specific reference intervals.

In Poland there are no published data on thyroid hormone concentrations in healthy pregnant women. The aim of our study was to establish trimester-specific reference intervals for TSH, fT_3 , and fT_4 in Polish pregnant women.

Material and Methods

The study included 172 pregnant women examined at 4 locations: Krakow, Bialystok, Poznan, and Warsaw. The median age was 35 years (range: 27–47, interquartile range: 33–39). All women were healthy, had no history of thyroid disease, were using the recommended iodine prophylaxis, and had normal thyroid-peroxidase antibodies (anti-TPO <50 IU/mL). Anti-TPO was only measured as it is considered as a more sensitive marker of thyroid autoimmunity, and so far there are not enough studies confirming the usefulness of measuring both anti-TPO and anti-TG (anti-thyroglobulin antibodies) [14].

We excluded patients with previously diagnosed thyroid disease, taking medication with proven impact on the function of the thyroid gland, with elevated TSH values in relation to the reference values for the general population and those that developed other illnesses during pregnancy. In addition, we retrospectively excluded from the group patients whose newborns had TSH >15 mIU/L in their screening test.

In all of the centers, there was approval from the ethics committee and the patients signed a consent for the study.

TSH, fT_3 , fT_4 , and anti-TPO serum concentrations were measured 3 times, i.e., in the first, second, and third trimesters of pregnancy. Some women did not come in the recommended time, so for some of them there is no data for all trimesters of pregnancy. Neonatal TSH concentrations were collected from a Polish national screening program of newborns that includes all newborns in Poland.

Table 1. Results of TSH, fT₃, and fT₄ in the study group in consecutive trimesters of pregnancy

	1st trimester (n = 172)	2nd trimester (n = 172)	3rd trimester (n = 152)
TSH, mIU/L	1.16 (0.400–1.805)	1.26 (0.81–2.01)	1.27 (0.77–1.93)
fT ₃ , pmol/L	4.88 (4.47–5.29)	4.14 (3.73–4.66)	4.04 (3.78–4.42)
fT ₄ , pmol/L	15.48 (14.24–17.43)	13.23 (12.09–14.40)	12.36 (11.24–13.81)

Values are presented as medians (lower–upper quartile).

Blood samples from pregnant women were collected in the morning, fasting, or after a light meal. The blood was centrifuged after complete clotting, after 30–40 min from collection. TSH, fT₃, fT₄, and anti-TPO antibodies in serum were measured with an electrochemiluminescence Elecsys analyzer, using reagent kits from Roche. Limits of quantification were 0.005–100 mIU/L for TSH, 0–50 pmol/L for fT₃, 0–100 pmol/L for fT₄, and 5–600 IU/mL for anti-TPO. Reference ranges for adults used in the laboratory in which the assay were performed were 0.3–4.5 mIU/L for TSH, 11–22 pmol/L for fT₄, 3.1–6.8 pmol/L for fT₃, and 0–50 IU/mL for anti-TPO.

Statistical Analysis

As the analyzed variables had nonnormal distributions ($p < 0.05$ in the Shapiro-Wilk and Anderson-Darling tests), data are presented as medians (lower–upper quartile). Also, because of the nonnormal distributions of the variables, nonparametric statistical tests were used, i.e., a Mann-Whitney test to assess the differences between the groups, and Friedman ANOVA to assess differences between the paired variables (repeated measures). The Spearman rank correlation coefficient was calculated to study associations between variables. Results were considered significant at $p < 0.05$. Reference intervals were assessed with the percentile method, using bootstrap, as recommended by the International Federation of Clinical Chemistry (IFCC) [15]. Outliers were detected by the Horn algorithm (with a ratio of 2) [15]. Before calculating the reference intervals, outliers were rejected (Fig. 1 shows the number of patients in each group after outlier rejection). The 2.5 and 97.5 percentiles were accepted as the limits of reference intervals. The software used for calculations was Statistica 10 (StatSoft, Tulsa, OK, USA) and RefVal 4.11 (HE Solberg, Oslo, Norway [16]).

Results

The concentrations of TSH, fT₃, fT₄, and anti-TPO in all studied patients in consecutive trimesters of pregnancy are shown in Table 1.

Changes in hormone levels in this group of patients are shown in Figure 2. The concentration of TSH was significantly lower in the first trimester compared to the 2nd and 3rd trimesters of pregnancy (median: 0.934, 1.505, and 1.360 mIU/L; $p < 0.0001$ Friedman test); TSH con-

Fig. 1. Flowchart of included/excluded pregnant women.

centrations in the 2nd and 3rd trimesters did not differ significantly. fT₃ concentration was significantly higher in the 1st trimester compared to the 2nd and 3rd trimesters (4.88, 4.04, and 3.94 pmol/L; $p < 0.0001$). fT₄ concentration was highest in the 1st trimester of pregnancy and decreased in subsequent measurements (15.47, 13.20, and 12.61 pmol/L; $p < 0.0001$).

Fig. 2. The concentrations of TSH (a), fT₃ (b), and fT₄ (c). Data is presented as medians, interquartile range (box), nonoutlier range (whiskers), and outliers (dots). Significant differences between repeated measurements are described in the text.

The concentrations of TSH measured in each trimester of pregnancy were strongly correlated (correlation coefficients: $R = 0.69$ between measurements carried out in the 1st and 2nd trimesters; $R = 0.85$ between the 2nd and 3rd trimesters; $R = 0.70$ between the 1st and 3rd trimesters, respectively; $p < 0.0001$ for all correlations). Similar but weaker correlations were observed for fT₃ ($R = 0.24$, $p = 0.008$; $R = 0.44$, $p < 0.0001$; $R = 0.32$, $p = 0.004$, respectively) and fT₄ concentrations ($R = 0.44$, $p < 0.0001$; $R = 0.56$, $p < 0.0001$; $R = 0.32$, $p = 0.003$, respectively).

Neonatal TSH concentrations did not correlate with the concentrations of TSH and thyroid hormones in the mothers; the exception was a weak negative correlation with 1st trimester fT₄ ($R = -0.18$, $p = 0.036$).

TSH concentrations higher than 2.5 mIU/L in the 1st trimester were observed in 12 (5%) patients. These patients, as compared with the rest of the group, also had higher concentrations of TSH in the 2nd (2.87 [2.19–3.22] vs. 1.25 [0.76–1.99]; $p < 0.0001$) and 3rd trimesters (2.90 [2.10–3.70] vs. 1.30 [0.75–1.94]; $p = 0.001$). However, the concentrations of fT₃ or fT₄ in these patients were not different from the rest of the group throughout the pregnancies. There were no differences between the described groups in the levels of thyroid hormones in the whole course of pregnancy ($p > 0.3$ for all comparisons). Also, the children born to women who had TSH above 2.5 mIU/L in the 1st trimester did not differ from

other newborns with regard to neonatal TSH concentrations (2.15 [1.39–2.67] vs. 2.23 [0.96–3.09] mIU/L; $p = 0.6$).

Reference intervals for TSH, fT₃, and fT₄ calculated for consecutive trimesters of pregnancy are shown in Table 2.

Discussion

We present trimester-specific reference intervals for TSH, fT₃, and fT₄ calculated for pregnant women in Poland.

In Poland, widespread iodine prophylaxis began in 1996, based on mandatory iodination of salt and baby formula. This program was later improved by recommending iodine supplementation in pregnant and breastfeeding women (100–200 mg of potassium iodide). This has provided a reduction in the incidence of goiters in children and has lowered the concentration of TSH in newborns [17, 18]. This is the first study on thyroid hormone concentrations during pregnancy after the beginning of iodine prophylaxis in the Polish population.

TSH

TSH concentrations are low in the first trimester of pregnancy and increase subsequently. In our study, TSH

Table 2. The reference ranges for TSH, fT_3 , and fT_4 in consecutive trimesters of pregnancy

	1st trimester ($n = 172$)	2nd trimester ($n = 172$)	3rd trimester ($n = 152$)
TSH, mIU/L	0.009 (0.008–0.010) 3.18 (2.96–3.83)	0.05 (0.01–0.11) 3.44 (3.05–3.77)	0.11 (0.03–0.19) 3.53 (2.94–3.94)
fT_3 , pmol/L	3.63 (3.43–3.71) 6.55 (6.23–6.84)	3.29 (3.16–3.41) 5.45 (5.20–5.64)	3.10 (2.92–3.26) 5.37 (5.04–5.55)
fT_4 , pmol/L	11.99 (11.52–12.15) 21.89 (20.84–23.92)	10.46 (10.34–10.67) 16.67 (16.03–17.20)	8.96 (8.63–9.62) 17.23 (16.20–18.32)

Lower and upper limits of the reference intervals (2.5 and 97.5 percentiles) are provided with 90% confidence intervals.

was significantly lower in the 1st trimester compared to the 2nd and 3rd trimesters of pregnancy, the concentration in the 2nd and 3rd trimesters did not differ significantly. TSH levels measured in consecutive trimesters of pregnancy were strongly correlated with each other. This is consistent with the results of other researchers who developed the reference intervals for pregnancy [19–22]. Our study shows that the upper limits of the reference for TSH are slightly higher than recommended by the Endocrine Society guidelines [11] in the 1st, 2nd, and 3rd trimesters of pregnancy (i.e., 3.177, 3.442, and 3.530 mIU/L, compared to the recommended 2.5 mIU/L in the 1st trimester of pregnancy and 3 mIU/L in the 2nd and 3rd trimesters, respectively). This is probably related to geographical location, current iodine prophylaxis in Poland, and other specific characteristics of the population.

Springer et al. [19] compared the reference intervals of TSH and fT_4 in early pregnancy in 216 Czech women using the 7 most popular methods of analysis. The upper reference limit for TSH was 3.81 (3.48–4.16) mIU/L, and in our study it was 3.177 (2.963–3.830) mIU/L. Both studies were done on the basis of the results obtained using reagent kits from Roche.

The study by Khalid et al. [20] investigating the reference intervals for thyroid function tests during pregnancy in women in Ireland ($n = 351$, mean age: 30 years) set the upper reference limit for TSH in the first trimester (12 weeks' gestation) at 3.0 mIU/L. They also used reagent kits from Roche.

It is also difficult to determine the lower limit of the reference for TSH, especially in the 1st trimester of pregnancy. As mentioned, the strong interference with hCG significantly reduces the concentrations of TSH in

this period, which is not synonymous with the diagnosis of hyperthyroidism. Many researchers who want to determine the lower limit adopt different exclusion criteria in their studies. In the Irish study [20], women with TSH <0.1 mIU/L were excluded only if the clinical diagnosis of hyperthyroidism was made, and the Czech study [19] excluded women with TSH <0.01 mIU/L and $fT_4 >23$ pmol/L, and/or elevated anti-thyroid peroxidase antibodies. In our study, for the determination of reference intervals we included pregnant women with normal anti-thyroid peroxidase antibodies and excluded hyperthyroidism. Respectively, the lower reference limit for TSH in the 1st trimester was 0.1 mIU/L in the Irish study [20], 0.25 mIU/L in the Czech study [19], and 0.009 mIU/L in our study. Decreased TSH levels during pregnancy must be interpreted by an experienced clinician, including other laboratory and clinical parameters.

TSH reference intervals in the 2nd and 3rd trimesters were higher, respectively 0.050–3.442 and 0.110–3.530 mIU/L, which is also described by other authors. However, the lower reference limit in the second and third trimester of pregnancy in our observations is still lower than in the general population.

fT_4 and fT_3

fT_3 concentrations were significantly higher in the 1st trimester compared to the 2nd and 3rd trimesters (median: 4.88, 4.04, and 3.94; $p < 0.0001$). fT_4 concentrations were highest in the 1st trimester of pregnancy and decreased in subsequent measurements (median: 15.47, 13.20, and 12.61; $p < 0.0001$). Our data are consistent with the observations in the Czech [19] and Irish [20] studies, in which the reference intervals for fT_4 in the first

trimester of pregnancy equaled 11.5–18.6 and 11–19 pmol/L, respectively. In our study, reference intervals calculated for Polish women in the 1st trimester were 11.99–21.89 pmol/L, and in subsequent trimesters 10.46–16.67 pmol/L and 8.96–17.23 pmol/L. The concentrations of free thyroxine decrease in consecutive trimesters of pregnancy. This is a result of the thyroid response to the growing level of thyroid-binding globulin, increased fetal demand for thyroid hormones in the 1st trimester of pregnancy, and the altered metabolism of thyroid hormones in the developing placenta in late pregnancy. A significant decrease in the level of fT_4 in late pregnancy for healthy pregnant women is a common phenomenon and some authors report that isolated hypothyroxinemia does not affect pregnancy and child development [23, 24]. However, there are studies that show hypothyroxinemia, especially in the early stages of pregnancy, is a predictor of lower motor and intellectual development and pregnancy outcomes [25–27].

Comparing the reference intervals for TSH and thyroid hormones is very difficult. In the studies we chose for the comparison, the researchers used the same laboratory methods and reagent kits as we did, and the populations analyzed in those studies were as close as pos-

sible to the Polish population. However, it is important to underline, as shown in the study of Bliddal et al. [21], that even in the same region, the use of gestational-age-specific reference ranges from different laboratories led to misclassification due to differences in the immunoassays used. Up to 100% of maternal fT_4 levels fell outside the other cohort's reference range despite similar TSH levels.

As recommended by the Endocrine Society, it is advisable to determine the reference intervals for pregnant women in a given geographical area. Our results confirm the validity of recommendations for developing own reference intervals for thyroid hormones in different countries. Ethnicity, a degree of nutritional deficiency, and/or iodine supplementation have a significant effect on thyroid function during pregnancy [14]. The results of our study will help in the proper care and treatment of Polish women with impaired thyroid function in pregnancy.

Disclosure Statement

The authors have nothing to disclose.

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