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Review Article

Challenges in Interpretation of Thyroid Function Tests in Pregnant Women with Autoimmune Thyroid Disease

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Physiological changes during gestation are important to be aware of in measurement and interpretation of thyroid function tests in women with autoimmune thyroid diseases. Thyroid autoimmune activity is decreasing in pregnancy. Measurement of serum TSH is the first-line screening variable for thyroid dysfunction also in pregnancy. However, using serum TSH for control of treatment of maternal thyroid autoimmunity infers a risk for compromised foetal development. Peripheral thyroid hormone values are highly different among laboratories, and there is a need for laboratory-specific gestational age-related reference ranges. Equally important, the intraindividual variability of the thyroid hormone measurements is much narrower than the interindividual variation (reflecting the reference interval). The best laboratory assessment of thyroid function is a free thyroid hormone estimate combined with TSH. Measurement of antithyroperoxidase and/or TSH receptor antibodies adds to the differential diagnosis of autoimmune and nonautoimmune thyroid diseases.

1. Introduction

Diagnosing maternal thyroid dysfunction during all stages of pregnancy is very important for the outcome for both mother and foetus [1, 2]. Women with hypothyroidism treated insufficiently with levothyroxine (high serum concentration of thyrotropin (TSH) or serum free thyroxine (T4) in the low normal range) deliver babies with significantly lower IQ and/or other inhibited neuropsychological development [3, 4]. Such offspring outcome has even been demonstrated in women with a serum concentration of T4 in the low normal range during pregnancy [5].

Prevalence of autoimmune thyroid disease (AITD) is high in women of reproductive age, whether or not they are pregnant [6]. AITD not only affects fertility [6], but may also lead to a decreased thyroid reserve with decreased availability of thyroxine. This is particularly important in the first half of pregnancy, in which the foetal development depends on the delivery of thyroxine from the mother [7, 8]. Although autoimmune thyrotoxicosis, Graves' disease, is rare in pregnant women, transfer of TSH receptor antibodies, which can be either stimulating or blocking, may give rise to foetal and neonatal thyrotoxicosis or hypothyroidism, respectively [9, 10].

As a natural consequence of the importance of thyroid hormones for foetal brain development much focus has been given to diagnosing both overt and subclinical (or mild) thyroid dysfunction as early as possible in pregnant women, recently resulting in international consensus guidelines [10]. Although the guidelines do not recommend universal screening of all pregnant women, most specialised clinical caretakers would attempt at including as many women as possible in a case finding programme. Women with autoimmune thyroid diseases or a family history of such belong to the risk groups [10].

Apart from general global problems in accomplishing this type of care due to financial and/or infrastructure restrictions, there are also many other reasons why these

efforts have limited success. One of them is associated with the biochemical measurements of thyroid function undergoing many complicated changes during pregnancy, and the corresponding issue of educating these important matters to the physicians who are caretakers of pregnant women. The question of whether precise detection and adequate treatment of thyroid insufficiency in pregnancy are feasible is still unanswered but recent progress and better insights into physiological changes, trimester-specific reference ranges, and intra- versus interindividual variability on the assessment of thyroid function in the single pregnant woman should give a better background for the future [11–13]. The present paper will focus on the choice of tests for assessment of biochemical thyroid function in pregnant women with AITD, together with their strengths and limitations. Information from two recent guidelines have been used in part as reference [10, 14] as well as the web-based textbook: www.thyroidmanager.org/ [15].

2. Physiological Changes during Pregnancy and Consequences for Thyroid Function Assessment

Normal pregnancy entails complicated and substantial changes in thyroid function [15]. The circulating thyroid hormone binding globulin (TBG) increases due to an oestrogen-induced increase in its production and at the same time the serum iodine decreases, the synthesis of thyroid hormones is increased, there are changes in the deiodinase activity, and, toward the end of the first trimester, when chorionic gonadotropin (HCG) levels are the highest, a significant fraction of the thyroid-stimulating activity is from HCG. Furthermore, thyroid autoimmune activity—reflected by thyroid autoantibody concentrations in serum—is usually decreasing due to a general immune suppressive action from the pregnancy, and finally plasma volume expands by approximately 50%, resulting in, for example, a lower serum albumin concentration. Serum concentrations of total T4 and T3 increase due to the increase of TBG. Serum concentrations of free thyroid hormones and TSH should physiologically be within normal limits, except in the short period of time when TSH may become suppressed due to the HCG effect (gestational hyperthyroidism). But it must be emphasised that normal reference ranges from a non-pregnant population are not to be considered "normal" in pregnancy. All the above-mentioned physiological changes, including the high TBG concentration, influence the laboratory measurements even of the free thyroid hormones. There is therefore a huge risk of false interpretation of thyroid function tests in pregnancy [16].

3. Biochemical Diagnosis of Thyroid Dysfunction in Pregnancy

3.1. Measurement of Serum TSH. The most sensitive method for screening for thyroid dysfunction in a healthy, non-pregnant population is the measurement of TSH serum concentration due to the log-linear relationship between TSH and free T4: even small changes in T4 concentrations will provoke very large changes in serum TSH. However, in pregnant women thyroid and pituitary functions are not stable, and, therefore, measuring TSH is not sufficient and often inappropriate for the assessment of thyroid function during gestation. If serum TSH measurement is used alone, the mother is likely to be insufficiently treated with levothyroxine for hypothyroidism or overtreated with antithyroid drugs for thyrotoxicosis, both of which resulting in maternal hypothyroidism, which in turn seriously affects the foetal brain development.

A typical example of such biochemical misdiagnosis during followup of antithyroid drug-treated Graves' disease is demonstrated in one of the cases from our tertiary referral department [17]. A 32-year-old 24-weeks pregnant woman was referred from a local hospital due to the finding of a large foetal goitre by routine scan. It was her second pregnancy, and she had been treated with antithyroid drugs for Graves' disease for 9 years. This included treatment during a previous pregnancy 5 years before, which resulted in a male baby with severely reduced cerebral capacity. Upon referral she was treated with 20 mg thiamazole daily. She had at the local hospital been considered sufficiently euthyroid based on a normal TSH of 2.9 mU/L (population-based reference range 0.4–4.0 mU/L), total T4 97 nmol/L (60–140 nmol/L), and free T4 7.8 pmol/L (7–20 pmol/L). She had a high level of TSH receptor antibodies at 24 U/L (<1.5 U/L). A more elaborate foetal ultrasound showed a male foetus with polyhydramnios and an enlarged thyroid gland with measures of $1.4 \times 3.5 \times 3.5$ cm, which was predominantly intrathoracic. Cord blood TSH was highly elevated at 34.5 mU/L, and free T4 was reduced to 13.8 pmol/L. The misdiagnosis of the thyroid function had been based primarily on the normal maternal levels particularly of TSH, which were, however, reflecting a delayed pituitary reaction to the slightly lowered free thyroid hormone levels. It is mandatory for doctors taking care of pregnant women with thyroid diseases to have a thorough knowledge of the evolution of the normal thyroid function during pregnancy as well as during treatment of thyroid dysfunction in order to avoid such unfortunate and unnecessary cases [17].

3.2. Measurement of Total or Free Thyroid Hormones. Measurement of the peripheral thyroid hormones themselves is complicated by a number of problems, the most important of which is the relationship to the gestation-induced elevation of the serum concentration of TBG. Since mostly immunoassays are used, biased values can derive from thyroid hormone antibodies in a woman with autoimmune thyroid disease, or heterophilic antibodies interfering in either the assays for TSH or thyroid hormones [18]. The elevated total hormone concentrations during gestation can display diverse reactions in the free thyroid hormone assays, either performing by giving a correct value or in most situations resulting in either over- or undercorrection. Consequently, results of free thyroid hormone measurements may very likely be either over- or underestimated leading to wrong diagnosis. A more reliable free thyroid hormone estimate is provided by measurement of total hormone concentrations (T3 and T4)

and correction for the increased binding proteins by either direct measurement of TBG (to provide T4/TBG or T3/TBG ratios) or a T3 or T4 uptake test. The latter can be calculated into free thyroid hormone indices, but as discussed in a very recent paper [19] these indices may also be incorrect during late pregnancy, probably due to insufficient correction of such extreme elevation of binding proteins, for which the methods are not designed. A qualitative or semiquantitative assessment of the total hormone concentrations and binding protein measurement separately may, however, be useful, and more so than the single free thyroid hormone results. It is important to note that the availability of these measurements depends on the local clinical biochemical laboratory.

From a clinical biochemical point of view total hormone measurements and creation of free thyroid hormone measurements are strictly the most reliable tests with the highest precision and accuracy. Very recently free thyroid hormones have been measured by equilibrium dialysis isotope dilution tandem mass spectrometry, which provides accurate, precise, fast, and simple measurements [20–24]. Such methods are, however, not generally available yet, and most laboratories still use immunoassays. In a recent paper, 3 immunoassays were compared with tandem mass spectrometry, and 2 of the 3 assays performed similarly to tandem mass spectrometry in late pregnancy, even when all 3 assays were dependant on binding proteins [19]. Thus, overall, the results of thyroid function testing during pregnancy are still puzzling and difficult and often even impossible to interpret.

4. Do Trimester-Dependent Reference Ranges Solve the Problem of Assessing Thyroid Dysfunction in Pregnancy?

Another problem in thyroid function tests is the population-based reference ranges, because they depend not only on the composition of the population and the iodine intake but also highly on the laboratory methods used. Therefore, there is a strong need of laboratory-dependent reference ranges, in order not to rely only on the reference range provided by the assay manufacturer. Because the progression of pregnancy and foetal, neonatal, and child health are dependent on adequate thyroid hormone supplementation throughout pregnancy, trimester-specific reference intervals for thyroid functions can be crucial for both maternal and foetal health. The physiologic changes associated with pregnancy require an increased availability of thyroid hormones by 40% to 100% to meet the needs of mother and foetus. Trimester-specific population-based reference ranges in order to correct for the physiological changes with increasing total hormone concentration and de- or increasing free hormones and suppressed TSH have been published from many sources in recent years [13, 24–37]. This approach will reduce the global variability of thyroid hormone assessment by approximately 6–18% [27], but it is important to emphasize that, for this to occur, the use of laboratory and population-specific ranges is crucial, since measurements by different methods in different populations do provide very different ranges (examples shown in Table 1). The table is not extensive but just examples of the most recent publications are shown. When producing trimester-specific reference ranges it is important that seemingly normal women with thyroperoxidase antibodies should not be included in the population [36].

5. The Problem of Population-Based Reference Ranges

Another, probably even more important, problem complicating the use of population-based reference ranges also in pregnant women is that each individual has its own genetic setpoint, as it has been shown by Feldt-Rasmussen et al. [12] and recently by Andersen et al. [38] in a nonpregnant population. In the initial studies when the methods for measurement of thyroid function had a lower sensitivity and higher imprecision the intraindividual coefficient of variation (CV%) was between 6 and 17% [12], also confirmed in a recent publication using more modern methods [39], while the interindividual CV% was 11 to 25%. A more relevant way of evaluating this is through an individuality assessment which was in these studies below 0.5, indicating that the thyroid function cannot be meaningfully assessed by the populations-based reference ranges [12, 13, 38–40]. Boas et al. [13] found a similar magnitude of variability in healthy pregnant women with an interindividual variability of 13–20% for both total and free T3 and T4, independent of gestational week, and an intraindividual variability of the same variables of 8–10%.

It is therefore very possible that also during pregnancy changes within the same woman are more important than the specific single measurement in relation to a specific reference range [13, 41]. In this case it applies also when using gestation-specific reference ranges, although the latter have to be considered also. This will result in a reduction of the total variability of the thyroid hormone function tests. The mentioned gestation-specific reference ranges should, however, be assessed in a given population with the given assays used in the laboratory and should not solely be based on information from the kit manufacturer, from the literature, or from another neighbouring laboratory, even if this laboratory uses the same assay.

6. Thyroid Autoantibodies

The measurement of thyroid autoantibodies in pregnant women is mainly useful to substantiate the probability of thyroid dysfunction in biochemically unclear cases, to ensure a correct differential diagnosis in case of maternal dysfunction, to predict the risk for development or deterioration of maternal thyroid dysfunction, and in few situations to predict for intrauterine and/or neonatal dysfunction [10, 14, 15]. Affection of the foetus and neonate is probably exclusively related to the presence and placental transfer of TSH receptor antibodies, which can also predict maternal hyperthyroidism in 60–70% of cases. When it comes to hypothyroidism the NHANES III study found almost similar prevalences of antithyroperoxidase and antithyroglobulin

Table 1: Trimester-specific reference ranges in various studies. Only a sample of studies is shown in order to exemplify the variety of values obtained in different populations of pregnant women and by different methods. Free thyroid hormone values are given—in some of the studies also total hormones have been measured together with T3 uptake to perform a free T4 index/estimate. Not all studies excluded pregnant women with thyroid autoantibodies.

			TSH			Free T4			Free T3		
		Method	1st trim	2nd trim	3rd trim	1st trim	2nd trim	3rd trim	1st trim	2nd trim	3rd trim
Boas et al. [13]	All trim Longitudinal	Roche Modular Elecsys	0.2–3.4	0.4–3.6	0.4–4.2	12–22	10–18	10–18	3.5–6.3	3.3–5.7	3.3–5.8
Cotzias et al. [28]	All trim Cross-sectional	ADVIA Centaur System	0–5.5	0.5–3.5	0.5–4.0	10–16	9–15.5	8–14.5	3–7	3–5.5	2.5–5.5
Dhatt et al. [32]	All trim/Arabs Cross-sectional	Abbott Architect	0.06–8.3	0.17–5.9	0.2–6.9	8.9–24.6	8.4–19.3	8.0–18.0	ND	ND	ND
Dhatt et al. [32]	All trim/Asians Cross-sectional	Abbott Architect	0.12–7.4	0.3–5.5	0.3–4.9	11.3–21.9	9.7–18.5	8.9–16.6	ND	ND	ND
Gilbert et al. [31]	1st trim	Abbott Architect	0.02–2.2	ND	ND	10–17.8	ND	ND	3.3–5.7	ND	ND
Gong and Hoffman [34]	All trim Cross-sectional	Roche Modular Elecsys	ND	ND	ND	11–19	9.7–17.5	8.1–15.3	ND	ND	ND
Lambert-Messerlian et al.* [24]	1st and 2nd trim Longitudinal	Immulite 2000	0.1–2.7	0.4–2.8	ND	0.9–1.4	0.8–1.3	ND	ND	ND	ND
Larsson et al. [37]	All trim Longitudinal	Abbott Architect	0.1–3.4	0.4–3.4	0.4–4.0	ND	ND	ND	ND	ND	ND
La'ulu et al. [30]	2nd trim Cross-sectional	Abbott Architect i2000SR	ND	0.1–3.3	ND	ND	9.1–15.4	ND	ND	3.8–6.0	ND
Marwaha et al. [29]	All trim Cross-sectional	Roche Modular Elecsys	0.6–5.0	0.4–5.8	0.7–5.7	12–19.5	9.5–19.6	11.3–17.7	1.9–5.9	3.2–5.7	3.3–5.2
Pearce et al. [36]	1st trim Cross-sectional		0.04–3.6	ND	ND		ND	ND	ND	ND	ND
Price et al. [33]	1st and 2nd trim/Asians Cross-sectional	Bayer Diagnostics ACS:180	0.6–1.3	1.0–1.8	ND	11.8–13.4	10.9–12.1	ND	ND	ND	ND
Price et al. [33]	1st and 2nd trim/Caucasian Cross-sectional	Bayer Diagnostics ACS:180	0.7–1.1	1.2–1.5	ND	12.0–12.8	11.2–11.8	ND	ND	ND	ND
Soldin et al. [35]	All trim Longitudinal	Tandem Mass Spectrometry	0.2–2.99	0.5–3.0	0.4–2.8	3.7–23.4	7.4–18.9	8.3–15.6	ND	ND	ND

TSH was measured in mU/L, free T4 in pmol/L, and free T3 in pmol/L. ND: not done. * the 5th and 95th percentile; free T4 in μg/L.

Table 2: What to do in clinical practice concerning thyroid function tests in pregnancy, when diagnosing hypo- or hyperthyroidism, respectively?

(i) Hypothyroidism:
(a) Serum TSH, evaluation respecting the gestation-induced suppression
(b) Measurement of antithyroperoxidase antibodies
(c) Sometimes measurement of TSH receptor antibodies and/or thyroglobulin antibodies
(d) Free T4 estimate
(1) "Direct measurement" with difficulty of interpretation
(2) Measurement of total T4 and T3 uptake test—more reliable
(3) Measurement of total T4 and correcting by 50% increase for pregnancy/TBG effect
(ii) Hyperthyroidism:
(a) Serum TSH, evaluation respecting the gestation-induced suppression
(b) Measurement of TSH receptor antibodies
(c) Free T4 estimate/free T3 estimate
(1) "Direct measurement" with difficulty of interpretation
(2) Measurement of total T4/T3 and T3 uptake test—more reliable
(3) Measurement of total T4/T3 and correcting the nonpregnant reference range by 50% increase for pregnancy/TBG effect

Adapted from: [10].

autoantibodies in this normal population. However, only antithyroperoxidase antibodies were significantly associated with hypothyroidism (as well as with hyperthyroidism), whereas antithyroglobulin antibodies were not [42].

7. Conclusions

In conclusion, in clinical practice doctors should use all available thyroid function tests relevant for the diagnosis of the AITD in question (see Table 2) to avoid the risk of false interpretations and the resulting potential irreversible damage to the foetal brain. Both total and free thyroid hormones are liable to false results during pregnancy, and the biochemical panel of measurements performed when there is a suspicion of hypo- and hyperthyroidism should therefore be supplemented with antithyroperoxidase antibodies, in case of suspected hypothyroidism [42], and TSH receptor antibodies, in case of suspected hypo- or hyperthyroidism, respectively [43]. TSH is insufficient as sole and first-line diagnostic variable due to the thyroid-pituitary instability during pregnancy and to suppression during the high peak of HCG at the end of the first trimester. Only women without thyroperoxidase antibodies should be included when producing trimester specific reference ranges. The finding of low intraindividual variation of the thyroid hormones in serum would speak in favour of using the woman's own evolution of the thyroid hormones when diagnosing hypo- or hyperfunction. Since this is for obvious reasons not always possible, the use of trimester-specific references may at least serve to reduce the variability and improve diagnostics.

Access to a broad spectrum of thyroid function tests must be considered a prerequisite for taking proper care of pregnant women with AITD. Due to the high TBG concentration, the best laboratory assessment of thyroid function also in AITD is a free thyroid hormone estimate—in hypothyroidism a free T4 estimate combined with TSH and in hyperthyroidism free T4 and T3 estimates combined with TSH. These free thyroid hormone measurements do not always correct completely for the binding protein abnormalities. Thus, if in doubt, samples should be measured in another laboratory with different platforms for free thyroid hormone measurements or combined with total hormone measurement and a T3 uptake test. Measurement of antithyroperoxidase, antithyroglobulin, and/or TSH receptor antibodies will add to the differential diagnosis between AITD and nonautoimmune thyroid disease. Thus, presence of TPO antibodies very often predicts the risk of hypothyroidism, and, in pregnant women with low TSH, hyperthyroidism will be predicted by TSH receptor antibodies in 60–70%.

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