TITLE: GESTATIONAL THYROID FUNCTION ABNORMALITIES IN CONDITIONS OF MILD IODINE DEFICIENCY: EARLY SCREENING VERSUS CONTINUOUS MONITORING OF MATERNAL THYROID STATUS

SHORT TITLE: Monitoring maternal thyroid function over gestation


Key words: iodine deficiency disorders, pregnancy, isolated maternal hypothyroxinemia, hypothyroidism, foetal brain development.

CORRESPONDING AUTHOR (and person to whom reprints should be addressed):
Francesco Vermiglio M.D.
Cattedra di Endocrinologia, Policlinico Universitario
Via Consolare Valeria 98125 Messina, ITALY
Tel & fax +39 090 2213185
e-mail: francesco.vermiglio@unime.it

Number of words: 2629 (main text) 241 (abstract)
Number of tables: 2
Number of figures: 2
Introduction

There is a known association between untreated maternal hypothyroidism and increased risk of several adverse outcomes for both mother and foetus at all stages of pregnancy (1,2). Moreover, children born to mothers who experience even mild thyroid insufficiency at early gestational stages may be at risk for neuro-motor and cognitive deficits (3-5). In view of the severity of these consequences, and the awareness that they may be successfully prevented by prompt therapeutic intervention, many have recommended that thyroid function screening be routinely performed in pregnant women (6-8). Conversely, other investigators recommend thyroid function evaluation only in symptomatic women or in those with a personal history of thyroid disease. (9-12). In the latest guidelines for the management of maternal thyroid dysfunction during pregnancy the authors conclude that recommending universal screening may be premature. Nonetheless, the expert panel does agree that maternal thyroid function testing is advisable in selected groups of women considered to be at high risk for thyroid disease (aggressive targeted case-finding) (13).

Whatever the strategy, i.e. universal screening or case-finding, there is broad agreement on the advisability of performing maternal thyroid function tests as early as possible after conception. Performing the tests at this early stage, however, could pose the risk that any maternal thyroid underfunction occurring later in gestation might not be picked up. In an attempt to assess this risk, we conducted an observational study on a cohort of consecutive pregnant women living in a mildly iodine-deficient (ID) area, whose thyroid function had been longitudinally monitored throughout gestation. The main aim of this study was, therefore, to evaluate the timing of maternal thyroid failure occurrence in mildly iodine-deficiency and ultimately assess whether or not maternal thyroid function testing performed at an early stage in gestation only is in fact appropriate in detecting maternal thyroid underfunction in pregnant women from mildly ID areas.
Subjects and Methods

Area studied, clinical monitoring programme and participants.

The study was carried out in a currently mildly iodine-deficient [median urinary iodine excretion (UIE) 99.7 µg/24h; goiter in schoolchildren 16.3%] area of North-eastern Sicily area that was previously classified as moderately-severely or moderately ID (1976-1980 UIE 25.5±23.6 µg/24h; goiter in schoolchildren 65%; 1989-1991 UIE 48.1±38.2 µg/24h; goiter in schoolchildren 31.7%) (14,15). A wide range of neuro-intellectual disorders have been reported in schoolchildren from the area and attributed to various degrees of maternal thyroid insufficiency occurring during gestation (3,15-17). Based on this evidence, we set up a monitoring programme to track maternal thyroid function over the full course of gestation and, later on, a programme of iodine prophylaxis aimed at preventing/correcting any maternal thyroid insufficiency occurring during gestation in order ultimately to avoid neuro-behavioural and intellectual disorders in progeny was offered on a voluntary basis (18).

All pregnant women living in this area are invited to participate in our prevention programme. Women are first sampled not later than week 12 at first trimester and twice more in the second and third trimester (at approximately 6 week intervals). Thyroid function testing includes serum free-T4 (FT4) and TSH determinations. In assessing maternal thyroid function, we refer to both serum FT4 and TSH internal trimester-specific reference intervals calculated in a cohort of consecutive long-term iodine-supplemented healthy anti-thyroperoxidase (TPO-Ab) antibody-negative pregnant women (18). At initial and final sampling TPO-Ab and anti-thyroglobulin (Tg-Ab) antibodies are also determined.

All women found to be subclinically or overtly hypothyroid throughout gestation are given substitutive levo-thyroxine (L-T4). Women with mildly isolated hypothyroinemia (serum FT4 values below the lower limit of the trimester-specific reference range and TSH concentrations within the trimester-specific reference range), are also given substitutive L-T4, in line with the purely experimental design of the study.

To date, 426 pregnant women have been referred to our outpatient clinic. For the purposes of this study, 108/426 women were ineligible because they were receiving L-T4 replacement or semi-suppressive therapy.
for post-surgical hypothyroidism or nodular goiter prior to becoming pregnant. A further 98/426 women did not fulfil inclusion criteria either because they were not enrolled at early pregnancy or did not complete the scheduled follow up. The remaining 220 women, who had never been tested for thyroid dysfunction prior to becoming pregnant, made up our sample study. These women were variously iodine-supplemented. Indeed, in addition to the regular use of iodised salt, more than one half of them also received multivitamin compounds specifically prepared for pregnancy and containing iodine (100-150 mcg).

The study was approved by the Ethical Committee of the “G. Martino” Polyclinic, Messina. Informed consent was obtained from all the women recruited.

Measurements

Maternal circulating TSH and FT4, TPO-Ab and Tg-Ab [electrochemiluminescence immunoassay (ECLIA)] were determined using commercial kits supplied by Roche Diagnostics, GmbH, D-68298 Mannheim. Precision profiles showed inter and intra-assay coefficients of variation <5% over the entire measurement range.

Statistical analysis

Unless otherwise specified data are expressed as mean±SD. Statistical analysis was performed using the chi-square, or Fisher exact tests when appropriate, for categorical data.

Results

The clinical and biochemical features at presentation of the 220 studied women are reported in table 1.

TPOAb and/or TgAb were detectable in 18/220 (8.2%) women.

THYROID FUNCTION ABNORMALITIES OVER GESTATION

Raised TSH with or without decreased FT4 [overt hypothyroidism (OH) or subclinical hypothyroidism (SCH)]
At initial observation, 205/220 (93.2%) women showed normal TSH values. The remaining 15/220 (6.8%) women had raised TSH (>2.3 mIU/liter), and four of them also had serum FT4 below the lowest trimester-specific reference range (11.9 pmol/liter) (figure 1, panel a). One third of these women (5/15) were anti-thyroid antibody positive.

During the 2nd trimester, a further 11 women showed TSH concentrations that were higher than the normal trimester-specific upper limit (2.8 mIU/liter), with abnormally low FT4 concentrations (<10.4 pmol/liter) for gestational age in 3 of them (figure 1, panels b and c). Anti-thyroid antibodies were positive in 3/11 (27.3%) hypothyroid women.

Finally, over the third trimester, in none of the women was TSH found to exceed the upper trimester-specific limit (3.0 mIU/liter) (figure 1, panels d and e).

Overall, overt or subclinical hypothyroidism affected 26/220 (11.8%) women, 8 of whom (30.7%) were anti-thyroid antibody positive.

**Isolated low serum FT4 concentrations and normal serum TSH [Isolated Hypothyroxinemia (IH)]**

At initial observation, 7/220 (3.2%) women had IH, with FT4 concentrations below the lower limit for gestational age but normal TSH levels (figure 1, panel a). Of these, only one was anti-thyroid antibody positive.

A gradual reduction in FT4 concentrations was observed in a high proportion of women over the course of the second trimester; these were found to fall below the lower limit in 28 women, despite TSH concentrations remaining consistently within the normal range (figure 1, panels b and c). Anti-thyroid antibodies were positive in 3/28 of these women.

Finally, during the 3rd trimester of gestation, 21 more women, none of whom were TPO and/or Tg Abs positive, displayed subnormal (<10.3 pmol/liter) FT4 concentrations (figure 1, panels d and e).

Overall, 56/220 (25.4%) women exhibited IH over the course of gestation and anti-thyroid antibodies were detectable in only 4/56 (7.1%) of them.
Overall, 82/220 women (26/82 with OH/SCH and 56/82 with IH) were given substitutive L-T4 (median thyroxine dose 1.65 µg/kg/day). In all of them, serum TSH and/or FT4 values returned to normal within 4 weeks and remained so during further follow up.

**OBSTETRICAL AND NEONATAL OUTCOME**

Data relative to obstetrical and neonatal outcomes were obtained for 204/220 women and are shown in Table 2. No differences in either gestational or neonatal parameters were observed in the women who were found to be consistently euthyroid (129/204) throughout gestation or in those who received substitutive L-T4 treatment for either OH/SCH (24/204) or IH (51/204).

**RISK OF OH/SCH IN ANTIBODY-POSITIVE PREGNANT WOMEN**

Of the 18/220 women who at presentation tested positive for TPO-Abs and/or Tg-Abs, 8/18 (44.4%) experienced OH/SCH, during either the 1st (5/8) or the 2nd (3/8) trimester. Of the 202/220 antibody-negative women, 18/202 (8.9%) were diagnosed with OH/SCH. Therefore, the relative risk (RR) of OH/SCH in antibody-positive women was 5.0 ($\chi^2$ 20.02, p<0.0005).

Of the remaining 10/18 antibody-positive women, 4/18 (22.2%) exhibited IH, mostly throughout the 2nd trimester, and 6/18 (33.3%) displayed no thyroid function abnormalities at any stage of gestation.

**TIMING OF OH/SCH OCCURRENCE AND POTENTIAL FOR MISDIAGNOSING AT EARLY THYROID FUNCTION TESTING**

The overall prevalence of OH/SCH over the course of gestation amounted to 11.8% (26/220). It is interesting to note that of those women experiencing hypothyroidism, just over half of them (15/26) were identified at presentation. In further follow up, either OH or SCH could be detected in the residual 11/26 at both early (6/11, between weeks 13 and 19) and late (5/11, between weeks 20 and 26) phases of the 2nd trimester. Consequently, 42.3% hypothyroid women would not have been diagnosed had we limited our observation to early thyroid function tests alone.

The frequency distribution of OH/SCH for each gestational period is shown in figure 2 (panel a).

As concerns IH, this mild condition of thyroid underfunction became progressively more common from the end of the first trimester onwards, peaking between weeks 20 and 26. Indeed, at presentation only 7 women
displayed FT4 values below the 2.5 percentile for gestational age, the remaining 49/56 (87.5%) dropped to this limit later in gestation (figure 2, panel b).

Discussion

Our study evaluated the timing of maternal thyroid failure in a cohort of pregnant women from a mildly ID area who had never been tested for thyroid dysfunction prior to becoming pregnant. The main objective of the study was to assess the benefit of thyroid function testing at early gestation only in identifying maternal thyroid dysfunction.

Very recently an ad hoc Endocrine Society committee established consensus guidelines which strongly recommend maternal thyroid function screening in selected groups of women considered to be at high risk for thyroid dysfunction, mainly because of a personal or family history of autoimmune disease (13). However, the efficacy of this strategy has recently been called into question, by the results of a prospective study, which concludes that testing only high-risk pregnant women would cause nearly one third of cases of hypothyroidism during early gestation to escape detection (19, 20). The recommendations further state that screening should consist of TSH measurement “performed before pregnancy when possible, or at the first prenatal visit” (13). This timing of intervention would certainly guarantee the prompt identification of women affected with thyroid insufficiency prior to becoming pregnant, but has the potential to overlook any women who might develop thyroid insufficiency in the subsequent stages of pregnancy. Indeed, pregnancy represents a challenge for the maternal thyroid, due to a progressive increase in hormone demand that can only be met by a very marked augmentation in hormone output. This end-point is ensured by physiological adaptations of the thyroidal economy, provided that the thyroid gland is fully operative and iodine intake adequate (21).

The women included in this study were variously iodine-supplemented. In addition to the regular use of iodised salt, more than one half of them also received multivitamin compounds containing 100-150 mcg of iodine. Median urinary iodine excretion at recruitment was consistent with mild iodine deficiency. About 8% of the women had detectable thyroid auto-antibodies. Overall, the prevalence of either overt or subclinical
hypothyroidism was nearly 12% and almost 60% of the hypothyroid women were identified at recruitment. The remaining 40% became hypothyroid in the further follow up, namely over the course of the 2nd trimester, and would not have been identified had we limited our observation to the first thyroid function test alone. Antithyroid autoantibodies were present in about one third of hypothyroid women (more or less equally distributed between the 1st and 2nd trimesters), and in a small percentage of women who displayed only minor thyroid abnormalities (isolated hypothyroxinemia). Overall, thyroid autoimmunity was associated with a 5-fold increased risk of hypothyroidism. Nonetheless, the vast majority of women experiencing either overt or subclinical hypothyroidism throughout gestation repeatedly tested negative for thyroid antibodies. This suggests that a feature other than thyroid autoimmunity, namely iodine deficiency, might play a major role in the occurrence of hypothyroidism in these women. It is reasonable to believe that the iodine needs of women experiencing thyroid underfunction throughout gestation were not fully met by their daily iodine intake and that the iodine stored in their thyroid gland was not sufficient to ensure adequate hormone synthesis and secretion for the whole gestational period. In other words, the occurrence of maternal thyroid insufficiency during the second trimester in women who proved euthyroid at very early testing might be explained by failure of the maternal thyroid to keep up with increased hormone demand due to an inadequate iodine supply. A similar mechanism might be involved in the occurrence of milder biochemical thyroid abnormalities, such as isolated hypothyroxinaemia, which in our series was observed in nearly one quarter of women, mainly from the end of first trimester onwards, although the cause of isolated hypothyroxinaemia is not fully understood. Epidemiological data from either moderately or mildly iodine deficient areas, have shown that a critical reduction in maternal free thyroxine, especially during early gestation, may not necessarily be matched by a proportional and simultaneous TSH increase. This is likely due to the fact that, over the first trimester, placental human chorionic gonadotropin stimulates the preferential IDI-related thyroidal output of T3, which, in turn, triggers negative feedback on pituitary TSH secretion. In these women, therefore, circulating T3 is normal or even slightly over the upper limit, TSH falls within the normal range, and the women are clinically euthyroid even when biochemically hypothyroxinemic (2). The causes of isolated hypothyroxinemia in women from iodine-sufficient areas (19, 22) are even less clear, though an
iodine intake that does not meet the requirements of pregnancy cannot be categorically ruled out in these women either. It remains to be demonstrated whether or not the condition is in fact consistent with potential thyroid failure. It has recently been shown that mild isolated hypothyroxinemia does not affect pregnancy outcome (23). Conversely, current clinical and experimental evidence seems to suggest that early and prolonged (until week 24) maternal hypothyroxinemia is a risk factor for impaired foetal brain development (3,22,24-26). Moreover, other reports of poor developmental outcome in preterm babies indicate that a normal supply of maternal T4 continues to have an important protective role after midgestation (27,28). Because of the potential irreversibility of foetal brain damage, we decided arbitrarily to give substitutive L-T4 treatment to women experiencing isolated hypothyroxinemia, in order to ensure FT4 levels similar to those observed in adequately iodine supplemented women at the same stage of pregnancy. We do not know whether or not this strategy of intervention improved obstetrical and neonatal outcomes, due to the lack of a control group of untreated hypothyroid/hypothyroxinemic women. Actually, no differences were observed in the pregnancy outcomes of either L-T4 treated or persistently euthyroid pregnant women or in their newborns. Nonetheless, this aspect was beyond the scope of our study, whose principal focus was on the timing of the occurrence of maternal thyroid underfunction. Accordingly, the main conclusion of our study is that in mildly ID areas thyroid function testing early in gestation is only partly effective in identifying thyroid dysfunction in pregnant women, because maternal thyroid underfunction also occurs later in gestation in apparently healthy women and in the absence of thyroid autoimmunity. Of course, a strategy of systematic screening and monitoring of thyroid function in all pregnant women from mildly ID areas should first be justified by cost-benefit analyses specifically designed to clarify this issue. Nonetheless, since iodine deficiency is the main cause of maternal thyroid insufficiency all over the world (29,30), basic prevention should consist of adequate and long-term iodine supplementation of women of child-bearing age prior to becoming pregnant, in order to permit the accumulation of sufficient iodine stores to meet the increased needs of both mother and foetus.
DISCLOSURE STATEMENT

The authors have nothing to disclose.

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.
References


10. Surks MI, Ortiz E, Daniels GH, Sawin CT, Col NF, Cobin RH, Franklyn JA, Hershman JM, Burman KD, Denke MA, Gorman C, Cooper RS, Weissman NJ. Subclinical thyroid disease: scientific review and guidelines for diagnosis and management. *JAMA* 2004 291 228-238.


maternal thyroid failure in conditions of mild iodine deficiency. *J Clin Endocrinol Metab* 2008 93 2616-2621


27. den Ouden AL, Kok JH, Verkerk PH, Brand R, Verloove-Vanhorick SP. The relation between neonatal thyroxine levels and neurodevelopmental outcome at 5 and 9 years in a national cohort of very preterm and/or very low birth weight infants. *Pediatr Res* 1996 39 143-145


30. WHO Secretariat on behalf of the participants to the Consultation. Andersson M, de Benoist B, Delange F, Zupan J. Prevention and control of iodine deficiency in pregnant and lactating women and in children less than 2-years-old: conclusions and recommendations of the Technical Consultation. *Public Health Nutr* 2007 102(12A) 1606-1611
Table 1
Characteristics of the studied pregnant women at recruitment (n=220)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>n (%) of total</th>
<th>mean±SD</th>
<th>median</th>
<th>range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (yrs)</td>
<td>220 (100)</td>
<td>--</td>
<td>29</td>
<td>17-40</td>
</tr>
<tr>
<td>Gestational age (wks)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 8 wk</td>
<td>57 (25.9)</td>
<td>10.1±1.8</td>
<td>10</td>
<td>5-12</td>
</tr>
<tr>
<td>9-12 wk</td>
<td>163 (74.1)</td>
<td>11.0±1.1</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>81 (36.8)</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>1</td>
<td>74 (33.6)</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>≥2</td>
<td>65 (29.5)</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>FT4 pmol/L</td>
<td>220 (100)</td>
<td>15.7±2.6</td>
<td>15.7</td>
<td>10.3-26.2</td>
</tr>
<tr>
<td>TSH mUI/L</td>
<td>220 (100)</td>
<td>1.06±0.97</td>
<td>0.85</td>
<td>0.01-6.5</td>
</tr>
<tr>
<td>TPO and/or Tg Abs positivity</td>
<td>18 (8.2)</td>
<td>--</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary Iodine Excretion (µg/L)</td>
<td>220 (100)</td>
<td>--</td>
<td>96</td>
<td>50-385</td>
</tr>
</tbody>
</table>
Table 2
Obstetrical and neonatal outcome (n=204)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>OH/SCH on L-T4 (n=24)</th>
<th>IH on L-T4 (n= 51)</th>
<th>Euthyroid (n=129)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preeclampsia % (n)</td>
<td>4.2 (1)</td>
<td>3.9 (2)</td>
<td>3.9 (5)</td>
<td>NS</td>
</tr>
<tr>
<td>Gestational week at delivery median (range)</td>
<td>39 (35-42)</td>
<td>39 (32-40)</td>
<td>39 (32-42)</td>
<td>NS</td>
</tr>
<tr>
<td>Preterm delivery % (n)</td>
<td>4.2 (1)</td>
<td>3.9 (2)</td>
<td>7.7 (10)</td>
<td>NS</td>
</tr>
<tr>
<td>Spontaneous delivery % (n)</td>
<td>41.7 (10)</td>
<td>62.7 (32)</td>
<td>68.2 (88)</td>
<td>NS</td>
</tr>
<tr>
<td>Cesarean section % (n)</td>
<td>58.3 (14)</td>
<td>37.3 (19)</td>
<td>31.8 (41)</td>
<td>NS</td>
</tr>
<tr>
<td>Stillbirths % (n)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.7 (1)</td>
<td>NS</td>
</tr>
<tr>
<td>Birth weight (g) M±SD (range)</td>
<td>3237±504 (2000-4400)</td>
<td>3332±635 (2100-4500)</td>
<td>3218±415 (1810-4120)</td>
<td>NS</td>
</tr>
<tr>
<td>Length (cm) M±SD (range)</td>
<td>49.4±1.7 (46-53)</td>
<td>50.2±2.7 (47-55)</td>
<td>50.1±1.3 (47-52)</td>
<td>NS</td>
</tr>
<tr>
<td>Head circumference (cm) M±SD (range)</td>
<td>33.9±1.7 (31.5-37)</td>
<td>33.6±1.2 (32-37)</td>
<td>34.5±0.9 (32.5-36)</td>
<td>NS</td>
</tr>
<tr>
<td>1-min Apgar Score median (range)</td>
<td>9 (5-10)</td>
<td>9 (8-9)</td>
<td>9 (3-10)</td>
<td>NS</td>
</tr>
<tr>
<td>5-min Apgar Score median (range)</td>
<td>10 (9-10)</td>
<td>10 (9-10)</td>
<td>10 (6-10)</td>
<td>NS</td>
</tr>
</tbody>
</table>
Figure 1.
Individual serum FT4 and TSH values over gestation.
**Figure 2**
Frequency distribution of overt/subclinical hypothyroidism and isolated hypothyroxinemia over gestation.

(a) Overt/Subclinical Hypothyroidism (%)
- 5-12 weeks (1st trimester)
- 13-19 weeks (2nd trimester)
- 20-26 weeks (3rd trimester)
- 27-33 weeks
- 34-term (post-term)

(b) Isolated Hypothyroxinemia (%)
- 5-12 weeks (1st trimester)
- 13-19 weeks (2nd trimester)
- 20-26 weeks (3rd trimester)
- 27-33 weeks
- 34-term (post-term)