Maternal Thyroid Function During Pregnancy and Behavioral Problems in the Offspring: The Generation R Study


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ABSTRACT: Maternal thyroid function during pregnancy is implicated in the neurodevelopment of the offspring, yet little is known about the effect of maternal thyroid parameters on the behavior of children. We investigated the association of maternal thyroid function during the first half of pregnancy with parent-reported problem behavior of the offspring up to age 3 y. In the Generation R study, a population-based cohort of 3736 children and their mothers, data on maternal thyroid function and child’s behavior were examined. The degree of internalizing and externalizing problems in the children was assessed with the Child Behavior Checklist at ages 1.5 and 3 y.

Maternal thyroid function [free thyroxine (T4), total T4, and TSH] was assessed before the 18th week of gestation for each pregnancy. In addition, they showed that children’s IQ score was inversely related to maternal TSH levels in mid gestation.

METHODS

Setting. This study was embedded in Generation R, a population-based cohort in Rotterdam from early fetal life onward (11,12). Mothers with a delivery date between April 2002 and January 2006 were enrolled. The study was approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam. Written informed consent was obtained from all adult participants.

Abbreviations: CBCL, The Child Behavior Checklist; DSM, Diagnostic and Statistical Manual of Mental Disorder; GEE, Generalized Estimating Equation; T4, thyroxine
**Subjects.** We measured one or more thyroid parameters in 4892 pregnant women before 18th week of gestation. Thirty-six pregnant women were excluded because of the current use of thyroid medication. Of the 4856 remaining women, 3369 mothers completed the Child Behavior Checklist (CBCL) for their children at the age of 3 y. The CBCL for the child was completed by 3177 mothers and 2656 fathers. In total, 3736 children (77%) with maternal thyroid and behavioral data were included in one or more analysis.

**Maternal thyroid parameters.** We assessed maternal thyroid parameters in pregnancy at the first prenatal visit. The GA at the time of blood sampling was <18 wk in all participants (mean = 13.3 wk, SD = 1.7). Within 24 h after sampling, the plasma was stored at −70°C. The TSH, free and total T4 were determined in the stored samples in batches over a 6-mo period, using chemiluminescence assays (Vitros ECI Immunodiagnostic System Ortho Clinical Diagnostics, Rochester, NY). Reference values of free and total T4 for nonpregnant women in our laboratory were 11–25 pmol/L and 58–128 nmol/L. The interassay and intra-assay coefficients of variation for free T4 were 4.7–5.4% and 1.4–2.7%. The respective coefficients for total T4 were 4.6–6.4% and 2.6–2.7%. To obtain pregnancy reference ranges, the normal population reference ranges for total T4 were multiplied by 1.5 as recommended by Endocrine Society Clinical Practice Guideline (6). The interassay and intra-assay coefficients of variation for TSH were 2.5–4.1% and 1.0–1.2%. Hypothyroxinemia was defined as TSH levels within the reference range for pregnancy (>0.03 mIU/L and <2.5 mIU/L) and a free T4 below the 10th percentile (6,13). This percentile corresponded to 11.76 pmol/L. In line with the Endocrine Society Clinical Practice Guideline (6) we defined maternal hypothyroxinemia as TSH plasma levels in the assessment of hypothyroxinemia (TSH levels >0.4 mIU/L or <4.0 mIU/L) (6,8). Thyroid parameters were measured after delivery, and parents were not informed about the results of the tests, except one clinical case that was excluded from this study.

**Child’s problem behavior.** To assess the child’s problem behavior, the CBCL for ages 1½–5 y was used (14). The CBCL consists of 99 items to which a standardized rating of behavioral and emotional problems of the children can be obtained. Two broad groupings of syndromes are scored: internalizing (anxiety, sadness, and withdrawn) and externalizing (attention problems and aggressive behavior). Five Diagnostic and Statistical Manual of Mental Disorder (DSM)-oriented scales consistent with DSM diagnostic categories were derived and used for additional analyses: affective, anxiety, peer problems, attention deficit/hyperactivity, and oppositional defiant problems. The CBCL is a valid instrument to measure the degree of behavioral and emotional problems of children at young age. The version used in this study is a revision of CBCL2–3 y and is specific for preschoolers but aims at a slightly wider age range than the previous version. It can be used to define the externalizing problems in children as young as 12 mo (15) and has been previously used to define the internalizing and externalizing problems at the age of 3 y (16). In addition, it is the only scale that explores the development of the child, measured by the Total Problems Scale of the CBCL/1½–5 y and the CBCL/4–18 y (14). Mesman and Koot (17) showed that the internalizing and externalizing problems at 2–3 y increased the risk of similar problems at 10–11 y and externalizing problems predicted DSM diagnosis of Attention Deficit/Hyperactivity Disorders and Oppositional Defiant Disorders. According to the CBCL manual, 98th percentile of the sample was used to define clinical cut-off (corresponding scores at 3 y: 8.4 for attention deficit/hyperactivity and 7.2 for oppositional defiant problems). Based on this cut-off, 79 children (2.1%) had attention deficit/hyperactivity problems at 1½ y. Only 18 children (0.5%) had scores above clinical cut-off for oppositional defiant problems at 1½ y. These numbers were 72 (2.3%) and 81 (2.2%) at 36 mo. These categories define children with high levels of attention deficit/hyperactivity and oppositional defiant problems and are not clinical diagnoses.

The CBCL was completed mostly by mothers when the children were 1½ y (mean age = 18.4 mo, SD = 1.0). Both mothers and fathers completed the CBCL again at the age of 3 y (mean age = 36.7 mo, SD = 1.4 and mean age = 36.9 mo, SD = 1.4). The correlation coefficients (r) between mother and father ratings of internalizing and externalizing scales at 3 y were 0.56 and 0.57. These numbers were very close to the mean correlation (r = 0.60) between two parents assessing the child’s emotion and behavior in the same setting reported in a review (18). The intraclass correlation coefficient (ICC) for mother reported CBCL at 1½ and 3 y was 0.72. The ICC for mother and father reported CBCL at 3 y was 0.73.

**Covariates.** We considered the following variables as potential confounders: Apgar score, GA and weight at birth, maternal psychopathology, cigarette smoking during pregnancy, and educational level (10,19–21). We also controlled for the GA at the time of thyroid assessments and child’s age when the CBCL was completed.

During enrollment, information on maternal age, parity, maternal educational level, and ethnicity of the child were obtained. Maternal smoking was assessed twice, at the time of enrollment and the 30th week of gestation. The Brief Symptom Inventory, a validated self-report questionnaire with 53 items, was applied to assess maternal psychopathology in mid pregnancy. We used the Global Severity Index as an indicator of psychopathological problems (22).

The child’s national origin was defined based on the origin of parents and grandparents and categorized into Dutch, Moroccan, Turkish, Surinamese, Antillean, other Western, or other non-Western ethnicity. Information on Apgar scores 1 and 5 min after birth, birth weight and mode of delivery was derived from medical records. GA of the child at birth was defined based on fetal ultrasound measures.

**Data analysis.** We used independent t tests, Mann-Whitney U tests and χ² statistics to compare characteristics of children included in the analysis with those who were excluded.

CBCL internalizing and externalizing scores were the dependent variables and analyzed primarily as continuous variables. First, we performed multiple linear regressions to assess whether maternal thyroid function is associated with the child’s internalizing and externalizing scores at the age of 1½ and 3 y. The determinants were maternal plasma levels of TSH, free and total T4. We divided them by their SD to enable comparison. We tested the normal distribution of the residuals in the analyses with TSH (23). To avoid multiple testing, we further explored our results only in case of an association with broadband scale (post hoc analyses). To this aim, we used the following DSM-oriented subscales of the CBCL: attention deficit/hyperactivity and oppositional defiant problems. Because of the small number of children with clinical attention deficit/hyperactivity and oppositional defiant problems in general population both subscales were transformed as continuous variable. Of the tested variables, only maternal age, educational level and psychopathology, child’s gender and ethnicity, mode of delivery and GA at the time of thyroid sampling were retained as confounders, based on the change-in-estimate method (5% change criterion) (24). Significance of quadratic terms of thyroid determinants was also examined because of possible nonlinear relationship (7,25).

CBCL scores, reported by two informants and at two time points, are correlated and assess the same construct. Therefore, we analyzed the overall outcome (mother- and father-report internalizing and externalizing scores at 1½ and 3 y), using a Generalized Estimating Equations (GEE) procedure to get to a more precise effect estimate and to reduce the error derived from multiple comparisons (type I error) (26). Any difference between two informants and a possible time trend are not easily interpretable in such a combined model.

**Nonresponse analyses.** Of 4856 pregnant women with data on thyroid parameters, 3736 completed the CBCL for their children. The children whose mother did not complete the CBCL (n = 1120, 23.1%) were more likely to have non-Dutch national origins [64.4 versus 34.5% for the children with CBCL data, x² = 290.2 (1), p < 0.001]. Mothers of nonresponders group were younger than mothers of the children with CBCL data (mean difference = 3.2 y; 95% CI = 2.9, 3.5; p < 0.001), less educated [42.3% primary level versus 16.0%, x² = 363.5 (2), p < 0.001], and were more likely to continue smoking during pregnancy [25.9 versus 14.9%, x² = 64.1 (2), p < 0.001].

**RESULTS**

The characteristics of the children and their mothers are summarized in Table 1. A total of 8.8% of the mothers fulfilled the criteria for hypothyroxinemia during early pregnancy. By using the alternative range for maternal TSH (0.4–4.0 mIU/L), this percentage changed to 9.8. The range for the thyroid parameters were as follows: TSH, 0.0–33.9 mIU/L; total T4, 63.3–380.0 nmol/L; and free T4, 6.4–94.6 pmol/L. The mean (SD) of attention deficit/hyperactivity and oppositional defiant problems at 3 y were 0.3 (2.3) and 2.9 (2.2). Maternal age was negatively associated with CBCL externalizing scores at 3 y (B = −3.7 per year of maternal age; 95% CI: −0.14, −0.05; p < 0.001). Non-Dutch national origin was associated with an increased risk of externalizing scores at 3 y (B = 1.93; 95% CI, 1.35, 2.51; p < 0.001). Mothers with only primary education had children with higher externalizing scores at 3 y (B = 1.79; 95% CI, 1.10, 2.49; p < 0.001).

In univariate analysis, there were no differences between the mean values of maternal plasma levels of TSH, free T4,
and total T4 in groups of children with and without attention deficit/hyperactivity or oppositional defiant problems (numbers are not shown).

Table 2 summarizes the association between maternal thyroid function and internalizing scores in the offspring. Maternal TSH was not associated with internalizing scores reported by mothers. Higher levels of TSH did not increase the risk of a high internalizing score in children, as demonstrated by the GEE approach using internalizing scores reported by father and mother at 1½ and 3 y ($B = 0.10$ per SD of TSH; 95% CI, −0.01, 0.21; $p = 0.07$). Similarly, free and total T4 did not predict the internalizing scores of children. Looking at 1½ and 3 y separately, we found that TSH levels were not associated with higher internalizing scores at 1½ and 3 y, reported by mothers ($B = 0.05$ per SD of TSH; 95% CI, −0.10, 0.20; $p = 0.52$ and $B = 0.01$ per SD of TSH; 95% CI, −0.16, 0.16; $p = 0.96$).

Table 3 shows the associations between maternal thyroid parameters and externalizing scores in children. Higher plasma levels of TSH were consistently associated with externalizing scores. Higher TSH levels increased the risk of a high externalizing score at 3 y as reported by fathers ($B = 0.26$ per SD of TSH; 95% CI, 0.02, 0.50; $p = 0.03$). But more importantly, analyses with GEE confirmed a positive association between TSH levels and externalizing scores ($B = 0.22$ per SD of TSH; 95% CI, 0.04, 0.40; $p = 0.02$), combining mother- and father-report externalizing scores at 1½ and 3 y. Maternal free and total T4 were not associated with children’s externalizing scores at 1½ and 3 y. Analysing mother-report externalizing scores at 1½ and 3 y separately showed that maternal TSH levels were associated with higher externalizing scores, but neither of these association was statistically significant ($B = 0.20$ per SD of TSH; 95% CI, −0.02, 0.42 and $B = 0.14$ per SD of TSH; 95% CI, −0.07, 0.36).

Comparing these findings with the results of GEE methods confirmed that pooling the scores from the reports of different informants in different times increases the precision of estimate as reflected by narrower CIs. The posthoc analyses showed that higher plasma levels of TSH were related to higher scores on attention deficit/hyperactivity ($B = 0.08$ per SD of TSH; 95% CI, 0.01, 0.15, and 0.05) and oppositional defiant problems (numbers are not shown).

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variables were tested as potential confounders but did not change the effect estimate (see the Methods and Materials section).

Maternal age, educational level and psychopathology, child’s gender, ethnicity, mode of delivery, and GA at the time of maternal thyroid sampling. Other variables were tested as potential confounders but did not change the effect estimate (see the Methods and Materials section).

Figure 1 illustrates the unadjusted association between TSH and externalizing behavioral mean scores in children at 3 y (father report), The Generation R Study. *Error bars are SE. **The children were divided in five equal groups based on 20th, 40th, 60th, and 80th percentile of maternal TSH.

Total of children in one or more analyses is 3736. B gives the estimate of increase in CBCL score per SD of thyroid parameters. Models were adjusted for maternal age, educational level and psychopathology, child’s gender, ethnicity, mode of delivery, and GA at the time of maternal thyroid sampling. Other variables were tested as potential confounders but did not change the effect estimate (see the Methods and Materials section).

* At the 1½ y assessment, <10% of informants were the primary caregivers other than mothers.
† SD of TSH, 1.45; SD of free T4, 3.48; and SD of total T4, 31.31 (SDs are calculated in whole sample).

Our results support the evidence that TSH is a good indicator of thyroid function problems because of the delicate feedback mechanism of pituitary. Mild increases in TSH, as a stimulatory mechanism for thyroid hormone secretion, can signal low levels of maternal thyroid hormones (27). These may lead to impaired fetal brain development and subsequent externalizing problems. However, from the results, we cannot infer that maternal TSH affects internalizing and externalizing scores differently. First, the effect estimates were very similar with largely overlapping confidence intervals. Second, the overall association between TSH and internalizing scores just failed to reach the significant level. We must be careful not to rule out an association between maternal thyroid parameters and attention deficit/hyperactivity and oppositional deviant problems in children.

DISCUSSION

In this study, higher plasma levels of maternal TSH in the first half of pregnancy predicted the externalizing scores in the offspring. An effect of maternal TSH on the internalizing scores was less clear but cannot be ruled out in this study. Plasma levels of free and total T4 in the mothers were not associated with internalizing and externalizing scores in their children.

Results from molecular (4,5) and clinical (7) observations provide evidence for a prominent role of thyroid hormones in brain development. Vermiglio et al. (8) showed that maternal hypothyroxinemia and TSH levels during pregnancy are associated with attention-deficit hyperactivity disorders in children. The retrospective design of the study and the small sample size make it difficult to infer a causal relationship from these results. Kooistra et al. (20) demonstrated that maternal free T4 but not TSH predicts the behavior of neonates. They assessed the behavior of the child at the age of 3 wk, which is too early to interpret the outcome as behavioral problems. In this study, we showed that maternal TSH can predict children’s externalizing scores. Further exploratory analyses extended this by showing an association between maternal thyroid parameters and attention deficit/hyperactivity and oppositional deviant problems in children.
GEE approach, we gained power. This is comparable with a significant effect sizes for maternal behavior in the general population impacts on fetal brain development, which determines behavioral and emotional problems later in life.

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