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Research Paper

Pregnancy-induced alterations in mitochondrial function in euthyroid pregnant women and pregnant women with subclinical hypothyroidism; relation to adverse outcome

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ABSTRACT

Background: It is well documented that overt hypothyroidism is associated with adverse pregnancy outcomes, but studies of subclinical hypothyroidism have demonstrated conflicting results.
Objective: Thyroid hormones are known to regulate mitochondrial function, and the aim of this study was to examine the possible relationship of subclinical hypothyroidism and mitochondrial dysfunction to adverse pregnancy outcomes in pregnant women.
Methods: Women in their third trimester of pregnancy ($n = 113$) who did not receive thyroid medication were included in this cross-sectional study. All participants were interviewed, and their thyroid status was determined. All participants had concentrations of thyroid hormones (fT_4 and tT_3) within the reference range. In addition to thyroid status, mitochondrial membrane potential (MMP) and reactive oxygen species (ROS) were measured by flow cytometry. To establish a reference range of MMP and ROS, a group of euthyroid, nonpregnant women were used as euthyroid controls. Adverse pregnancy outcome was defined as preterm delivery, preeclampsia, placental abruption, Apgar score <7 points 1 minute after birth, or postpartum hemorrhage.
Results: The prevalence of subclinical hypothyroidism among pregnant women was 17% ($n = 19$), and the number of overall adverse pregnancy outcomes was increased ($p = 0.02$) compared with that in euthyroid pregnant women. Preeclampsia, poor Apgar score, and postpartum hemorrhage were more frequent in the subclinical hypothyroidism group than in the euthyroid group ($p = 0.04$, $p = 0.001$ and $p = 0.03$, respectively), and more women showed prolonged gestation and gave birth later than 41 weeks of gestation than in the euthyroid group ($p = 0.04$). Compared with euthyroid, nonpregnant controls, a physiological upregulation of mitochondrial function was observed in euthyroid pregnant women. This was impaired in pregnant women with subclinical hypothyroidism. Compared with euthyroid, nonpregnant controls, pregnant women had increased ROS regardless of their thyroid status.
Conclusion: We speculate that the unfavorable effects on mitochondrial function in women with subclinical hypothyroidism may be associated with higher prevalence of adverse pregnancy outcomes.

Abbreviations: TSH, thyroid-stimulating hormone; TPOAb, thyroid peroxidase antibody; fT_4, free thyroxine; tT_3, total triiodothyronine; TMRM, tetramethylrhodamine methyl ester; ROS, reactive oxygen species; PBMC, peripheral blood mononuclear cells; MMP, mitochondrial membrane potential; carboxy-H2DCFDA, 5(6)-carboxy-2'-7'-dichlorodihydrofluoresceindiacetate; GA, gestational age; BMI, body mass index.

Conflicts of interest statement: The authors declare no conflicts of interest.

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Introduction

Subclinical hypothyroidism is defined as increased level of thyroid-stimulating hormone (TSH) and levels of thyroid hormones within the reference range. Subclinical hypothyroidism is the most frequent thyroid disease during pregnancy, compared with overt hypothyroidism and overt and subclinical hyperthyroidism [1]. Depending on the cut-off values used for the definition of subclinical hypothyroidism, ethnicity, and study design, the reported prevalence varies between 1.5% and 4% [2–5]. It is well documented that overt hypothyroidism is associated with increased risk of obstetric complications such as miscarriage, gestational hypertension, preterm delivery, stillbirths, and perinatal deaths [6–8]. However, although studies of maternal subclinical hypothyroidism have also suggested a relation to adverse effects [9–11], this relation is not as well established.

A number of cellular functions are regulated by thyroid hormones, and one major function is that of mitochondrial energy production and biogenesis [12]. Several studies have reported impaired thyroid hormone-regulated mitochondrial function in patients with subclinical hypothyroidism [13–15].

The aim of the present study was to examine pregnancy outcomes in women with subclinical hypothyroidism, and the relation to a possible mitochondrial dysfunction, as well as to examine whether mitochondrial function was influenced by pregnancy.

Material and methods

Women in their third trimester of pregnancy who consulted the Department of Obstetrics, Naestved Hospital, Region Zealand, Denmark, between June 2012 and January 2013 were included.

Women with known thyroid disease, diabetes, and those receiving any medical treatment for thyroid disease were excluded. In total, 115 pregnant women (all of Caucasian ethnicity) were included. Two women were subsequently excluded because they gave birth at home and their data were unavailable. The remaining 113 data sets were complete.

All participants were interviewed to ensure full investigation of their obstetric history. Body mass index (BMI) before pregnancy was obtained from general practitioner records.

All women were examined during the first trimester, and gestational age (GA) was determined by ultrasound. GA at delivery, placental abruption, preeclampsia, cesarean section, postpartum hemorrhage, Apgar score, birth weight, and any severe event occurring during the period from inclusion in the study until delivery were obtained from hospital records.

Adverse pregnancy outcome was defined as preterm delivery (GA <37 weeks), preeclampsia, placental abruption, Apgar score <7 points 1 minute after birth, postpartum hemorrhage (>500 mL), or delivery later than 41 weeks after gestation. Preeclampsia was defined as persistent blood pressure ≥140/90 mm Hg accompanied by proteinuria >300 mg/24 h or ++ proteinuria determined by urinary dipstick analysis (Multistix 7®, Siemens Healthcare, Tarrytown, NY, USA). The definition of adverse pregnancy outcome was based on former studies of hypothyroidism during pregnancy, and all criteria carry potential risks of increased mortality and morbidity.

To ensure that the control group of age-matched, euthyroid, nonpregnant women ($n = 42$) had not consulted the hospital regarding emergency or chronic disease, participants were recruited from a population study performed at Naestved Hospital during the same period [16]. The control group was included to establish the normal reference range of mitochondrial membrane potential (MMP) and production of reactive oxygen species (ROS). All laboratory tests were performed at the same laboratory using the same assays, and all nonpregnant euthyroid women had values of TSH, thyroid peroxidase antibody (TPOAb), and thyroid hormones within the reference ranges.

Biochemical variables

Measurements of TSH and thyroid hormones fT_4 and tT_3 were performed using an electrochemical luminescent immunoassay (Roche Cobas 6000, Basel, Switzerland). TPOAb was measured by KRYPTOR antiTPOn (BRAHMS, Hennigsdorf, Germany), detection limit 10 U/mL.

Normal values for thyroid hormones followed the standard references used at the Central Laboratory of Naestved Hospital: $fT_4 = 10.0–26.0$ pmol/L and $tT_3 = 1.20–2.80$ nmol/L. This reference range also covers the trimester-specific alterations (decrease of fT_4 and rise of tT_3 within the reference range).

Subclinical hypothyroidism was defined as raised serum concentration of TSH ≥3.40 mU/L (this cutoff value was chosen based on recommendations from the testing laboratory at the hospital) and thyroid hormones within the reference range. Thyroid peroxidase antibody positivity was defined by the cutoff value of TPOAb >60 U/mL.

Measurement of mitochondrial function

MMP and ROS production were measured using flow cytometry as previously described [15,17,18]. In brief, peripheral blood mononuclear cells (PBMC) were obtained from a freshly drawn blood sample and stained with 0.1 μmol/L tetramethylrhodamine methyl ester (TMRM) or 1 μM 5(6)-carboxy-2'-7'-dichlorodihydrofluoresceindiacetate (carboxy-H2DCFDA) (Invitrogen A/S, Taastrup, Denmark). The cells were incubated in the dark for 30 min at room temperature for TMRM and at 37 °C for carboxy-H2DCFDA. The cells were chilled on ice and immediately measured by flow cytometry (Accuri C6, BD Biosciences, Franklin Lakes, NJ, USA). A total of 20,000 leukocytes were acquired, and the lymphocytes were identified on the forward- and side-scatter parameters. The median TMRM and carboxy-H2DCFDA fluorescence intensities of PBMC were calculated using BD Accuri C6 software, and values were given as relative emission (arbitrary units, a.u.). The intra-assay variation of TMRM and carboxy-H2DCFDA measurements was <12%.

Because of the time-consuming procedure of performing flow cytometry on fresh blood specimens, the number of participants was limited.

Statistics

The Student's *t*-test or Mann–Whitney test was used to compare cases and controls. The Shapiro–Wilk test was used to test normality of the distribution of data, and the Wilcoxon signed-rank test was used to compare paired data. The discontinuous variables were compared using χ^2-test. The Spearman correlation coefficient was used to evaluate the correlation of the variables. A value of $p < 0.05$ was considered statistically significant. All statistical analyses were performed using STATA version 11 for Windows (StataCorp, College Station, TX, USA).

Ethical considerations

The study was approved by the Regional Research Ethics Committee of Zealand, Denmark (Reg. no. RVK SJ-294). The clinical trial registration at ClinicalTrial.gov is NCT01335802.