Previous Live Births and Induced Abortions May Precede Later Development of Graves' Hyperthyroidism

Allan Carlé^{a, b} Nils Knudsen^c Torben Jørgensen^{d, e} Bettina Thuesen^d
Jesper Karmisholt^{a, b} Stine Linding Andersen^{a, f} Inge Bülow Pedersen^{a, b}

^aDepartment of Endocrinology, Aalborg University Hospital, Aalborg, Denmark; ^bDepartment of Clinical Institute, Aalborg University, Aalborg, Denmark; ^cDepartment of Endocrinology I, Bispebjerg Hospital, University of Copenhagen, Copenhagen, Denmark; ^dCentre for Clinical Research and Prevention, Capital Region of Denmark, Copenhagen, Denmark; ^eDepartment of Public Health, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark; ^fDepartment of Clinical Biochemistry, Aalborg University Hospital, Aalborg, Denmark

Keywords

Hyperthyroidism · Thyrotoxicosis · Case-control study · Risk factor · Pregnancy · Live births · Parity · Abortion · Oestrogens · Contraceptives · Epidemiology · Population-based study

Abstract

Objective: To investigate the association between reproductive history and later development of various nosological subtypes of overt hyperthyroidism. **Study Design:** From the Danish population, we included incident hyperthyroid women, and for each case we recruited 4 euthyroid age-sex-region-matched controls from the same sub-population. Hyperthyroid cases/controls were: Graves' disease (GD, $n = 232/928$), multinodular toxic goitre (MNTG, $n = 91/364$), solitary toxic adenoma (STA, $n = 21/84$). Patients diagnosed with hyperthyroidism within 1 year after delivery including post-partum GD were excluded. In multivariate conditional regression models (reference: no reproductive events), we analysed the association between development of GD/MNTG/

STA and reproductive factors such as age at menarche/menopause, reproductive span, number of pregnancies/childbirths/abortions, investigations for infertility, and years on oral contraceptives. We adjusted for possible confounders such as alcohol intake, smoking, co-morbidity, and education. Age was studied as a potential effect measure modifier. **Results:** GD patients diagnosed before the age of 40 years had given births more often than control subjects (OR [95% CI] for 1/2/3+ births [ref.: nulliparous] were 1.57 [0.80–3.11]/2.06 [1.001–4.22]/3.07 [1.50–6.26]), and they had induced abortions performed more often (OR for 1/2+ induced abortions [ref.: no: events] were 0.99 [0.54–1.84]/2.24 [1.12–4.45]). No associations were observed between any reproductive factor and the development of MNTG or STA. **Conclusions:** Childbirths and induced abortions may be followed by development of Graves' hyperthyroidism after the post-partum period. This was not the case for the non-auto-immune subtypes of hyperthyroidism.

© 2018 European Thyroid Association
Published by S. Karger AG, Basel

Introduction

Hyperthyroidism is a common disease affecting women more often than men [1]. As first described by Parker et al. in 1961 [2], pregnancy is accompanied by a fall in thyroid antibody concentrations. Most [3, 4], but not all studies [5], have shown a lower risk during pregnancy of developing autoimmune thyroid disease such as Graves' hyperthyroidism and autoimmune hypothyroidism. Due to the so called "rebound phenomenon," the risk of autoimmune thyroid disease after delivery is higher than before pregnancy [3, 6–8]. The enhanced risk of Graves' hyperthyroidism up to 12 months post-partum is well known [5, 9] but it is not known whether this risk extends more than a year after delivery [10]. Age at menarche [11–13], age at menopause [12, 13], and the use of oral contraceptives (OC) [11, 12] have all been associated with altered risk for autoimmune diseases. A few studies examined the association between delivery and later development of Graves' hyperthyroidism [5, 10, 14, 15], but no study has extensively investigated the long-term repercussions of multiple reproductive factors on the risk of developing various types of hyperthyroidism. We previously observed a strong association between the number of pregnancies and the later development of autoimmune hypothyroidism [16]. Various autoimmune diseases may respond differently to previous pregnancy exposures [6]. Thus, we hypothesise that autoimmune Graves' thyrotoxicosis, as was the case for spontaneous autoimmune hypothyroidism, also may develop after the 1-year post-partum period. In the present study, we aimed to investigate if a similar association exists in autoimmune hyperthyroidism due to Graves' disease (GD) as well as in the non-autoimmune types of hyperthyroidism (multinodular toxic goitre [MNTG] and solitary toxic adenoma [STA]). In addition, we investigated whether other reproductive factors, such as infertility, use of OC, and years on menstruation had impact on the risk of later hyperthyroidism.

Subjects and Methods

The DanThyr (The Danish Investigation on Iodine Intake and Thyroid Diseases) monitoring programme was launched in 1997 to monitor thyroid disease before and after iodine fortification of salt. The fortification became mandatory in 2000 [17] but was effectively introduced in year 2001. We registered all women with incident hyperthyroidism diagnosed in a population using a prospective monitoring of all thyroid function tests performed. The study period spanned from March 1997 to December 2000. This encompassed 1,055,608 years of observation. For case-control analyses, we recruited euthyroid subjects randomly selected from the same population.

Patients

Two geographical areas were defined for the present study; an area in and around the city of Aalborg with moderate iodine deficiency (population, $n = 311,102$; of these, 155,957 were women; urinary iodine excretion = 45 $\mu\text{g/L}$ in subjects taking no mineral supplements [18]) and an area of Copenhagen with only mild iodine deficiency ($n = 227,632$, of these 119,419 were women; urinary iodine excretion = 61 $\mu\text{g/L}$ [18]). A surveillance register [19] was linked to databases at the four diagnostic laboratories responsible for all thyroid function testing performed in the two study areas (one in Aalborg, three in Copenhagen). The register identified all subjects with a first-time suppressed serum TSH in combination with a high T4 and/or T3 estimate. Every subject with possible new hyperthyroidism was individually evaluated to verify or disprove new overt hyperthyroidism [1]. Hyperthyroidism was classified into various nosological subtypes [1], but only hyperthyroid women diagnosed with GD, MNTG, and STA were considered for the present study. GD was defined in case of positive thyroid-hormone receptor antibodies (TRAb) and/or a diffuse technetium (Tc) uptake scan; STA and MNTG in case of a single versus multiple hyperfunctioning nodules on a Tc scintiscan. Patients who had given birth within 1 year prior to diagnosing hyperthyroidism were not included in the present study but were classified as suffering from post-partum thyroid dysfunction including post-partum Graves' hyperthyroidism [1].

In the study period (Fig. 1), we identified 398/350/46 women with GD/MNTG/STA (total, $n = 794$). In selected periods with staff available, we invited 516 women newly diagnosed with hyperthyroidism ($n = 311/180/25$, mostly patients <70 years of age) to participate in a comprehensive investigation program as described in detail previously [20]. Of those invited, 353 (68.4%) accepted participation.

We have previously in detail described the surveillance program, the use of a register linked to laboratory databases, the precise diagnostic criteria used to identify patients with overt hyperthyroidism, the algorithm for final verification of incident autoimmune hyperthyroidism, and the classification of hyperthyroidism into various subtypes of disease [1, 19].

Controls

By use of the Civil Registration System, in which all Danish inhabitants are registered with a unique identification number [21], we randomly extracted civilians residing in the two study areas for two cross-sectional studies. For each hyperthyroid patient, we included 4 randomly selected women from two surveys, all matched on age and residency. Survey I, a cross-sectional population study totalling 3,712 women, was performed in the two study areas Aalborg and Copenhagen in 1997–1998 [22]. Women aged 18–22, 25–30, 40–45, and 60–65 years participated. Control subjects outside these age categories were invited from the same population in 1998 (survey II; women, $n = 530$). The inclusion criteria for controls were a serum TSH between 0.2 and 5.0 mU/l and no previous thyroid disease. Each person fulfilling these criteria was considered a control subject (Fig. 1). We were able to find 1,056 female controls (survey I, $n = 660$; survey II, $n = 396$) matching the 343 women diagnosed with overt hyperthyroidism (GD/MNTG/STA; cases, $n = 232/91/21$; controls, $n = 928/364/84$). The healthy women were allowed to act as control subjects in more than one regression model. We were not able to identify 4 matching controls for 9 of the hyperthyroid women, and these cases were excluded from the study.

Fig. 1. Flowchart depicting the origin and selection of cases ($n = 344$) identified and their matched controls (4:1) simultaneously recruited from the same geographical area. Total numbers of controls used were 1,056, of which 776 were used for one analysis, and 280 acted as control for two or all subtypes of hyperthyroidism. The number of matched controls (*) used in regression analysis split by subtype of disease is given in the figure.

Investigational Program

Patients and controls underwent the same investigations and answered identical questionnaires. Participants had their height and weight measured, and BMI (body mass index) was calculated as weight measured in kg/(height in meters)². A blood sample was drawn at investigation and stored at -20°C . At study end, all samples were thawed and analysed in random order.

Questionnaires

We asked for the total numbers of pregnancies, live births, abortions (induced or spontaneous), the use of OC before menopause, age at first menstruation (menarche) and menopausal age. The reproductive span was defined as total years of menstruations (time period from menarche to menopause). The hyperthyroid patients were asked if they had given birth within a year prior to the diagnosis.

Participants filled out questionnaires on alcohol consumption (units per week) and smoking habits (never, previous, or current

smoking). We asked for cardiovascular co-morbidity (history of acute myocardial infarction, angina pectoris, cardiac arrhythmia, hypertension, or cerebral stroke) and non-cardiovascular co-morbidity (epilepsy, diabetes mellitus, asthma, chronic obstructive pulmonary disease, or gastrointestinal ulcer). Educational status was answered into five categories: basic school with no vocational education, vocational education up to 2 years, 3–4 years vocational education, vocational education for more than 4 years, and under vocational education.

Blood Specimen Analyses and Assay Characteristics

Thyroid peroxidase antibody (TPOAb), thyroglobulin antibody (TgAb), and TRAb were measured after study end [20, 23]. Subjects with antibody concentration above the functional sensitivity given by the manufacturer (TPOAb: >30 kU/L, TgAb: >20 kU/L, TRAb: >1 U/L) were regarded as antibody-positive (TPOAb+, TgAb+, TRAb+). TSH was measured in serum drawn from controls. Serum TSH, T3, and T4 concentrations at the date

Table 1. Baseline characteristics of hyperthyroid cases (at time of diagnosis) and their age- and region-matched controls

	Graves' hyperthyroidism		Multinodular toxic goitre		Solitary toxic adenoma	
	cases (n = 232)	controls (n = 928)	cases (n = 91)	controls (n = 364)	cases (n = 21)	controls (n = 84)
Age ^a	42.0 (33.0–52.7)	42.0 (30.5–53.7)	57.8 (51.4–66.5)	58.8 (50.9–65.8)	55.0 (49.5–65.6)	55.0 (50.7–65.6)
Region ^b						
Aalborg	160 (69.0)	640 (69.0)	76 (83.5)	304 (83.5)	20 (95.2)	80 (95.2)
Copenhagen	72 (31.0)	288 (31.0)	15 (16.5)	60 (16.5)	1 (4.8)	4 (4.8)
Weight ^a , kg	64.4 (57.2–73.9)	66.0 (59.7–73.8)	68.7 (60.1–76.4)	67.6 (59.8–75.6)	64.5 (50.4–74.8)	66.5 (54.2–77.5)
Height ^a , cm	166 (161–171)	166 (162–170)	163 (158–167)	164 (159–168)	161 (159–166)	165 (159–169)
BMI ^a , kg/m ²	23.2 (20.9–27.6)	23.9 (21.7–26.8)	26.2 (23.0–25.0)	25.0 (22.6–28.6)	24.6 (20.2–28.6)	24.4 (21.7–29.6)
TPOAb ^a , kU/L	202 (19–1,912)***	<30	<30	<30	<30	<30
>30 kU/L	171 (75.0)***	147 (16.0)	15 (16.7)	79 (21.9)	4 (19.0)	23 (27.4)
TgAb ^a , kU/L	208 (<20–83)***	<20	<20	<20	<20	<20
>20 kU/L	116 (51.5)***	134 (14.6)	20 (22.5)	71 (19.5)	3 (14.3)	15 (17.9)
TRAb ^a , kU/L	8 (3.9–14)	– ^d	<1	– ^d	<1	– ^d
>1 kU/L	209 (92.1)	– ^d	0	– ^d	0	– ^d
Pregnancies ^c	555 (2.39)***	1,851 (1.99)	268 (2.95)	956 (2.62)	47 (2.24)	221 (2.63)
Live births ^c	402 (1.73)**	1,337 (1.44)	223 (2.45)	748 (2.05)	39 (1.86)	178 (2.12)
Abortions ^c						
All	153 (0.66)	514 (0.55)	45 (0.49)	208 (0.57)	8 (0.38)	43 (0.51)
Induced	90 (0.39)	295 (0.32)	23 (0.25)	97 (0.27)	7 (0.33)	22 (0.26)
Spontaneous	63 (0.27)	219 (0.24)	22 (0.24)	111 (0.30)	1 (0.05)	21 (0.25)
Education ^b						
≤ vocation +2 years	147 (63.4)	547 (58.9)	7.4 (82.2)*	257 (70.6)	17 (85.0)	60 (71.4)
> vocation +2 years	85 (36.6)	381 (41.1)	16 (17.6)	107 (29.4)	3 (15.0)	24 (28.6)
Smoking ^b						
Current	97 (41.8)	354 (38.1)	45 (50.0)*	125 (34.3)	10 (50.0)	29 (34.5)
Previous	44 (19.0)	178 (19.2)	22 (24.4)	102 (28.0)	3 (15.0)	23 (27.4)
Never	91 (39.2)	396 (42.7)	23 (25.6)	137 (37.6)	7 (35.0)	32 (38.1)
Alcohol ^a , units per week	2 (0.5)***	4 (1–8)	1 (0–3)***	4 (1–8)	2 (1–6)	4 (2–7)
Co-morbidity ^b						
All	102 (44.0)*	335 (36.1)	56 (61.5)*	172 (47.3)	12 (57.1)	36 (42.9)
Cardiovascular	68 (29.3)***	180 (19.4)	40 (44.0)*	120 (33.0)	9 (42.9)	23 (27.4)
Non-cardiovascular	34 (14.7)	155 (16.7)	16 (17.6)	54 (14.3)	31 (14.3)	13 (15.5)

Three types of hyperthyroidism were investigated (Graves' hyperthyroidism, multinodular toxic goitre, and solitary toxic adenoma). Missing values: weight ($n = 9$), height ($n = 9$), BMI ($n = 10$), TPOAb ($n = 13$), TgAb ($n = 16$), TRAb ($n = 0$ for cases, no controls had TRAb measured), education ($n = 2$), smoking ($n = 2$), alcohol (no answer, $n = 2$; could not be specified for all types of beverages, $n = 38$). Levels of significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ for comparison between cases and controls within each of the three strata (subtype of hyperthyroidism). Continuous variables not Gaussian distributed were compared by Mann-Whitney U test. Categorized variables were compared by the χ^2 test. ^a Values are medians (interquartile range [25–75% percentiles]). ^b Values are numbers (percentage). ^c Values are numbers (per capita). ^d Not measured.

of diagnosis were registered for all hyperthyroid patients. Assay details have been described previously [19, 24].

Statistical Analysis

We used IBM Statistical Package for Social Sciences version 15.0 (SPSS, Chicago, IL, USA) for all calculations and statistical analyses. Data not normally distributed were expressed with medians and interquartile ranges (IQR, 25% and 75% percentiles). Groups of subjects were compared using Mann-Whitney U test, Pearson's χ^2 test, Fishers exact test, or independent t test. Associations between the primary reproductive factors (pregnancies, live births, abortions) and later development of hyperthyroidism were analysed in conditional uni- and multivariate logistic regression mod-

els. Furthermore, we explored the possible role for other reproductive factors such as age at menarche and menopause, reproductive span, infertility investigations, and the use of OC. Associations were expressed as odds ratios (OR) with 95% confidence intervals (95% CI). p values less than 0.05 or an OR with 95% CI not including 1.0 were regarded as statistically significant. Conditional regression analysis requires no missing values, and the 105 out of 16,060 (0.65%) questions regarding reproductive data left unanswered by participants were filled out by means of nearest neighbour hot-deck imputation [25]. In the regression models, we tested for multi-collinearity. In all analyses, the various reproductive factors were tested against circumstance with no reproductive event present. Tests for linear trend were calculated using the ordinal categories for

Table 2. Reproductive characteristics of hyperthyroid cases (at time of diagnosis) and their age- and region-matched controls

	Graves' hyperthyroidism		Multinodular toxic goitre		Solitary toxic adenoma	
	cases (<i>n</i> = 232)	controls (<i>n</i> = 928)	cases (<i>n</i> = 91)	controls (<i>n</i> = 364)	cases (<i>n</i> = 21)	controls (<i>n</i> = 84)
Pregnancies ^a						
0	39 (17.0)**	204 (22.0)	7 (7.9)	36 (9.9)	2 (11.1)	13 (15.5)
1	28 (12.2)	169 (18.2)	12 (13.5)	40 (11.0)	1 (5.6)	6 (7.1)
2	61 (26.5)	242 (26.1)	21 (23.6)	113 (31.0)	8 (44.4)	23 (27.4)
≥3	102 (44.3)	312 (33.7)	49 (55.1)	175 (48.1)	7 (38.9)	42 (50.0)
Age at 1st pregnancy ^b	23 (21–26)	24 (21–27)	22 (19–25)	23 (20–26)	23 (21–25)	22 (19–25)
Live births ^a						
0	59 (25.7)**	273 (29.4)	9 (10.1)	46 (12.6)	2 (11.1)	14 (16.7)
1	30 (13.0)	194 (20.9)	14 (15.6)	56 (15.4)	2 (11.1)	7 (8.3)
2	88 (38.3)	301 (32.5)	31 (34.8)	149 (40.9)	11 (61.1)	34 (40.5)
≥3	53 (23.0)	159 (17.2)	35 (39.3)	113 (31.0)	3 (16.7)	29 (34.5)
Abortions, induced ^a						
0	169 (73.5)*	701 (75.6)	69 (80.2)	283 (77.7)	13 (68.4)	68 (81.0)
1	36 (15.7)	168 (18.1)	11 (12.8)	66 (18.1)	5 (26.3)	12 (14.3)
≥2	25 (10.9)	58 (6.3)	6 (7.0)	15 (4.1)	1 (5.3)	4 (4.8)
Abortions, spontaneous ^a						
0	184 (79.3)	746 (80.4)	75 (82.4)	276 (75.8)	17 (89.5)	66 (78.6)
1	37 (15.9)	150 (16.8)	12 (13.2)	70 (19.2)	2 (10.5)	15 (17.9)
≥2	11 (4.7)	26 (2.8)	4 (4.4)	18 (4.9)	0	3 (3.6)
Age at menarche ^b	13 (12–14)	13 (13–14)	13 (12–14)	14 (13–15)	14 (13–15)	14 (13–15)
Menopause ^a	74 (32.9)	286 (30.9)	60 (79.3)	265 (73.0)	13 (68.4)	58 (69.0)
Age at menopause ^b	50 (46–51)	49 (45–51)	49 (45–51)	50 (46–52)	49 (47–50)	49 (45–52)
Reproductive span ^b	27 (19–35)	28 (17–34)	34 (30–38)	35 (31–38)	34 (31–37)	34 (26–37)
Investigated for infertility ^a	21 (9.1)	104 (11.2)	8 (9.1)	38 (10.6)	2 (10.5)	7 (8.6)
OC						
Ever use ^a	17 (76.6)	751 (81.1)	48 (53.9)	236 (65.0)	14 (73.7)*	49 (58.3)
Years on OC ^b	4 (0–10)	5 (1–10)	1 (0–7)	2 (0–8)	7 (0–15)	1 (0–6)

Three types of hyperthyroidism were investigated (Graves' hyperthyroidism, multinodular toxic goitre, and solitary toxic adenoma). Missing value: pregnancies (*n* = 8), live births (*n* = 8), age at 1st pregnancy (*n* = 22), induced abortions (*n* = 10), spontaneous abortions (*n* = 2), age at menarche (*n* = 21), menopause (*n* = 16), age at menopause (*n* = 59), reproductive span (*n* = 7), investigated for infertility (*n* = 25), oral contraceptives (OC) use (*n* = 7), years on OC (*n* = 43). Levels of significance: * *p* < 0.05, ** *p* < 0.01 for comparison between cases and controls within each of the three strata (subtype of hyperthyroidism). Continuous variables not Gaussian distributed were compared by Mann-Whitney *U* test. Categorized variables were compared by χ^2 test or Fischer's exact test. ^a Values are numbers (percentage). ^b Values are medians (interquartile range, 25–75% percentiles).

pregnancies, live births, and abortions as continuous variables. We adjusted for potential confounding by alcohol consumption (units consumed per week as a continuous variable), smoking history (never, current, and previous smoking), all cause co-morbidity (ever/never), and education (basic school plus up to 2 years of vocational schooling [e.g., store employees, carpenters, or mason] vs. more). We explored whether age or infertility interacted with the results. Age for GD/MNTG/STA patients was dichotomised at 40/55/55 years, which were the ages closest to the reported median ages divisible by 5 years.

Results

Baseline Characteristics of Cases and Controls

Median age at disease onset was: 42.0 years in GD, 57.8 years in MNTG, and 55.0 years in STA (Table 1). Patients diagnosed with nodular toxic goitre (MNTG and STA) had more co-morbidity probably due to their higher age at disease onset.

Table 3. Uni- and multivariate logistic regression models calculating the association between various reproductive factors and later development of the common types of hyperthyroidism (Graves' hyperthyroidism, multinodular toxic goitre, and solitary toxic adenoma)

	Graves' hyperthyroidism (GD)		Multinodular toxic goitre (MNTG)		Solitary toxic adenoma (STA)	
	univariate	multivariate	univariate	multivariate	univariate	multivariate
Pregnancies						
Trend ^a , <i>p</i> value	0.002	0.07	0.58	0.54	0.50	0.25
1	0.998 (0.58–1.72)	0.89 (0.51–1.53)	1.48 (0.53–4.12)	1.67 (0.57–4.93)	0.72 (0.061–8.54)	0.52 (0.030–9.13)
2	1.68 (1.01–2.77)*	1.52 (0.92–2.52)	1.03 (0.41–2.59)	0.92 (0.35–2.42)	1.77 (0.36–8.72)	2.19 (0.38–12.6)
≥3	2.16 (1.33–3.53)**	1.90 (1.15–3.12)*	1.42 (0.60–3.36)	1.15 (0.46–2.84)	0.74 (0.14–3.96)	0.61 (0.09–4.01)
Live births						
Trend ^a , <i>p</i> value	0.001	0.003	0.45	0.83	0.29	0.11
1	0.82 (0.49–1.37)	0.71 (0.42–1.19)	1.28 (0.51–3.22)	1.07 (0.40–2.82)	1.64 (0.17–15.5)	1.01 (0.076–13.5)
2	1.75 (1.10–2.79)*	1.57 (0.98–2.51)	1.12 (0.50–2.52)	0.93 (0.40–2.17)	1.92 (0.35–10.5)	2.53 (0.41–15.5)
≥3	2.13 (1.26–3.61)**	1.79 (1.05–3.06)*	1.66 (0.73–3.77)	1.23 (0.51–2.94)	0.53 (0.076–3.58)	0.40 (0.052–3.06)
Abortions, induced						
Trend ^a , <i>p</i> value	0.053	0.036	0.29	0.022	0.57	0.61
1	0.89 (0.60–1.33)	0.81 (0.54–1.22)	0.66 (0.33–1.29)	0.36 (0.17–0.78)*	1.91 (0.58–6.36)	1.92 (0.51–23.4)
≥2	1.76 (1.07–2.91)*	1.76 (1.05–2.93)*	1.54 (0.55–4.31)	1.37 (0.46–4.04)	1.09 (0.11–11.3)	1.42 (0.087–23.4)
Abortions, spontaneous						
Trend ^a , <i>p</i> value	0.33	0.44	0.40	0.63	0.67	0.78
1	0.96 (0.65–1.42)	1.03 (0.69–1.53)	0.64 (0.33–1.24)	0.71 (0.35–1.43)	0.49 (0.10–2.32)	0.56 (0.11–2.80)
≥2	1.70 (0.83–3.46)	1.61 (0.78–3.35)	0.84 (0.28–2.55)	1.03 (0.32–3.35)	no subjects	no subjects

In all models, no events were reference (OR = 1). In the multivariate models, the explanatory variables were: all-cause morbidity (categorized), educational status (categorized), smoking history (categorized), and alcohol consumption (continuous). ^a Trend for odds ratios in the regression model. Levels of significance: * *p* < 0.05, ** *p* < 0.01.

Reproductive History and Hyperthyroidism

GD patients, but not MNTG or STA patients, had more pregnancies, live births, and induced abortions compared to controls (Table 2). No differences between patients and controls were observed in terms of reproductive span, infertility, or the OC use in any of the three subtypes of hyperthyroidism.

Women diagnosed with GD more often reported pregnancies, live births, and induced abortions (Table 3). In terms of nodular toxic goitre (MNTG+STA), no associations to any reproductive exposures were found, suggesting no role for live births or abortions as predictors for later disease development. In GD, age modified the associations and results are showed dichotomised by the age of 40 years (Fig. 2). Among younger patients with GD diagnosed before the age of 40 years, the odds for having ≥3 pregnancies (OR 3.07 [1.50–6.26]), ≥3 live births (OR 3.06 [1.31–7.15]), and ≥2 induced abortions (OR 2.24 [1.12–4.45]) shows the age-dependent findings. On the other hand, no associations were found between these reproductive risk factors and the development of GD after the age of 40 years.

In the present study, 59 (25.9%) of 228 GD women reported no childbirths. Of those, only 8 (13.6%) had been

investigated for infertility. Autoimmunity may be associated with both infertility and the development of GD. However, introduction of previous infertility investigation in the multivariate models did not reveal any interaction (*p* = 0.44).

Discussion

In a Danish population-based case-control study, women newly diagnosed with Graves' hyperthyroidism in the years following the post-partum period had higher odds for previous pregnancies, live births, and induced abortions. Notably, associations were confined to women younger than 40 years. Furthermore, previous live births or abortions did not associate with hyperthyroidism of non-autoimmune origin.

Pregnancies and Live Births

Previous reports do not show equivocal associations between parity and thyroid disease development. A positive association between childbirth and the hospital referral of patients diagnosed with Graves' hyperthyroidism was suggested by Jørgensen et al. [6] (OR 1.19 [1.14–

Fig. 2. Associations between reproductive events (pregnancies, live births, induced abortions) and development of Graves' hyperthyroidism split by the age of 40 years. In the group of younger GD women (filled dots), OR for the events were (reference: 0 events): 1/2/≥3 pregnancies, 1.57 (0.80–3.11)/2.06 (1.001–4.22)/3.07

(1.50–6.26); 1/2/≥3 live births, 1.29 (0.68–2.45)/1.70 (0.84–3.42)/3.06 (1.31–7.15); 1/≥2 induced abortions, 0.99 (0.54–1.84)/2.24 (1.12–4.45). No significant findings were found in women aged 40+ years.

1.24)). In a Swedish study, Jansson et al. [10] reported an association between post-partum GD and repeated parities with a relative risk increase from 4.4 (2.2–9.1) in uniparous women to 8.1 (3.8–17) in those who had given birth to more than 2 children. Strieder et al. [26] reported 6.9 (1.5–31) higher odds for previous pregnancies among Graves' patients. Benhaim Rochester and Davies [27] studied 152 women newly diagnosed with GD at the age of 18–39 years and reported that 45% of all cases were diagnosed within the 1-year post-partum period, in which they observed a 2.1 increased relative risk. Unfortunately, they did not explore the risk profile among the 55% of the women diagnosed with GD in the subsequent years. Effraimidis et al. [28] reported a clear association between parity and the development of autoimmune hypothyroidism ($p = 0.006$), but only showed a trend in post-partum Graves' hyperthyroidism ($p = 0.06$). Finally, Rogers et al. [29] found no association between live births and a combined term of various autoimmune diseases, among them Graves' hyperthyroidism and Hashimoto's hypothyroidism.

Abortions

In the present study, women diagnosed with Graves' hyperthyroidism had more often experienced induced abortion compared to controls, whereas spontaneous abortion had no impact on later GD. Spontaneous abor-

tions are often caused by mutations or other metabolic failures at a very early stage. Thus, the hormonal and immunological disruptions may be less pronounced. On the other hand, the initial phase of pregnancy is normal in women having an induced abortion performed. This may explain the difference in the risk profile of spontaneous and induced abortions in the present study.

Pathomechanisms Involved

In our study series, repeated pregnancy and live birth exposures were associated with higher risk of autoimmune thyroid disease both in terms of autoimmune hypothyroidism [16] and in Graves' hyperthyroidism. It is, however, a notable finding that the risk differs substantially. We previously demonstrated OR for development of autoimmune hypothyroidism around 10/30/50 for 1/2/3+ live births (reference: nulliparous) [16]. The reported associations for GD were statistically significant, but with much smaller OR around 2–3 for previous live births. Both autoimmune hypothyroidism and GD are characterised by intrathyroidal lymphocytic infiltration [30], production of thyroid auto-antibodies, and subsequently altered function of the B- and T-cell function. However, hypothyroidism is believed to be caused by cellular immunity (Th1), whereas GD follows humoral immunity (Th2). This may be the reason for the large difference in risk in our studies of later autoimmune hypothyroidism versus Graves' hy-

perthyroidism following pregnancies and live births. In addition, patients diagnosed with post-partum Graves' thyrotoxicosis were excluded.

Microchimerism, i.e. the presence of fetal progenitor cells within the female body [31] may play a role in later development of autoimmunity in the mother. The presence of those genetically dissimilar cells has been demonstrated in early pregnancy [32] but also up to 38 years after delivery [33]. If pathophysiologically relevant, such cells may only play a crucial role for about a decade after delivery, as we found no association between live births and later hyperthyroidism after the age of 40 years.

Several studies claimed that a major part of Graves' patients are diagnosed within the post-partum period [10, 14, 27]. Most patients were diagnosed during the last half of the post-partum period, as there is a shift from Th1 to Th2 balance around 4–6 months after delivery, which is illustrated by rebound of B-cells peaking around 7–10 months after delivery [34, 35]. We now demonstrate that the increased risk is still there after the 1-year post-partum period. The lacking association between pregnancy exposures and both disease entities of nodular thyrotoxicosis illustrates that autoimmune mechanisms may have taken place.

In our study, some of the patients may have been undiagnosed for several months, which at least partially may have contributed to a late diagnosis in the months after the 1-year post-partum period. Thus, even if we attempted to follow the accepted definition of the post-partum period to be 1 year, we may have included some cases from an "extended" 12- to 18-month post-partum period or perhaps even longer.

Strengths and Limitations

The main strength of the present study is that information and referral bias is minimal [36]. We scrutinised hospital records, and searched for all TRAb measurements and scintiscans, and asked to complete disease record and reproductive history when participants joined our comprehensive programme in order to individually classify the specific subtype of hyperthyroidism using strict criteria. We included all overt hyperthyroid patients as they were diagnosed in the population, whenever diagnosed at primary care or after hospital referral [36].

One limitation is the case-control study design, as association does not allow for causation. In addition, it is often difficult to know whether one condition may lead to another or the reverse. However, several of Hill's criteria such as biological plausibility, temporality, and dose dependency are met in the present study [37]. We have

also statistically adjusted for a wide range of possible confounders.

Another limitation of our study is the 68.4% participation rate. Among those invited, some self-selection may have taken place. Of all patients, we invited mainly individuals aged up to 70 years and only in selected periods with staff available for assisting the comprehensive investigations.

We used nearest neighbour hot-deck imputation in the multivariate regression model to compensate for missing information mostly on alcohol intake. Raw data regression excluding subjects with any imputed value provided OR similar to those depicted in our models (data not shown).

In conclusion, it seems that various pregnancy exposures (live births and induced abortions) may be associated with enhanced risk for development of Graves' hyperthyroidism even after the post-partum period. Thus, thyroid function testing should always be considered in women consulting their physician in the first years after having given birth.

Acknowledgements

We are highly indebted to the general practitioners in Copenhagen and Northern Jutland, to the four clinical chemical laboratories at Aalborg Hospital, Bispebjerg Hospital, Frederiksberg Hospital, and the Laboratory of General Practitioners in Copenhagen for their collaborative support.

Statement of Ethics

The study was approved by Regional Ethics Committees in North Jutland and Copenhagen. Registry permission was obtained from the Danish Data Protection Agency. All participants gave written consent.

Disclosure Statement

The authors declare no conflicts of interest that may be perceived as prejudicing the impartiality of the research reported.

Funding Sources

This study was part of DanThyr, and it was supported by the following grants: IMK General Foundation; The Danish Council for Independent Research; Ministry of Food, Agriculture and Fisheries; the Danish Agency for Science, Technology and Innovation, Institute for Clinical Medicine, University of Aarhus; and Aase og Ejnar Danielsens Foundation.

References

- Carlé A, Pedersen IB, Knudsen N, Perrild H, Ovesen L, Rasmussen LB, et al. Epidemiology of subtypes of hyperthyroidism in Denmark: a population-based study. *Eur J Endocrinol*. 2011 May;164(5):801–9.
- Parker RH, Beierwaltes WH, Elzinga K, Spafford NR, Hassoun S. Thyroid antibodies during pregnancy and in the newborn. *J Clin Endocrinol Metab*. 1961 Jul;21(7):792–8.
- Davies TF. The thyroid immunology of the postpartum period. *Thyroid*. 1999 Jul;9(7):675–84.
- Editorial: immunological changes in women. *Lancet*. 1974 May;1(7863):909–10.
- Andersen SL, Olsen J, Carlé A, Laurberg P. Hyperthyroidism incidence fluctuates widely in and around pregnancy and is at variance with some other autoimmune diseases: a Danish population-based study. *J Clin Endocrinol Metab*. 2015 Mar;100(3):1164–71.
- Jørgensen KT, Pedersen BV, Nielsen NM, Jacobsen S, Frisch M. Childbirths and risk of female predominant and other autoimmune diseases in a population-based Danish cohort. *J Autoimmun*. 2012 May;38(2-3):J81–7.
- Amino N, Mori H, Iwatani Y, Tanizawa O, Kawashima M, Tsuge I, et al. High prevalence of transient post-partum thyrotoxicosis and hypothyroidism. *N Engl J Med*. 1982 Apr;306(14):849–52.
- Amino N, Miyai K, Onishi T, Hashimoto T, Arai K, Ishibashi K, et al. Transient hypothyroidism after delivery in autoimmune thyroiditis. *J Clin Endocrinol Metab*. 1976 Feb;42(2):296–301.
- Weetman AP. Immunity, thyroid function and pregnancy: molecular mechanisms. *Nat Rev Endocrinol*. 2010 Jun;6(6):311–8.
- Jansson R, Dahlberg PA, Winsa B, Meirik O, Säfwenberg J, Karlsson A. The postpartum period constitutes an important risk for the development of clinical Graves' disease in young women. *Acta Endocrinol (Copenh)*. 1987 Nov;116(3):321–5.
- Pedersen M, Jacobsen S, Klarlund M, Pedersen BV, Wiik A, Wohlfahrt J, et al. Environmental risk factors differ between rheumatoid arthritis with and without auto-antibodies against cyclic citrullinated peptides. *Arthritis Res Ther*. 2006;8(4):R133.
- Costenbader KH, Feskanich D, Stampfer MJ, Karlson EW. Reproductive and menopausal factors and risk of systemic lupus erythematosus in women. *Arthritis Rheum*. 2007 Apr;56(4):1251–62.
- Phillips DI, Lazarus JH, Butland BK. The influence of pregnancy and reproductive span on the occurrence of autoimmune thyroiditis. *Clin Endocrinol (Oxf)*. 1990 Mar;32(3):301–6.
- Tada H, Hidaka Y, Tsuruta E, Kashiwai T, Tamaki H, Iwatani Y, et al. Prevalence of postpartum onset of disease within patients with Graves' disease of child-bearing age. *Endocr J*. 1994 Jun;41(3):325–7.
- Rotondi M, Pirali B, Lodigiani S, Bray S, Leporati P, Chytiris S, et al. The post partum period and the onset of Graves' disease: an overestimated risk factor. *Eur J Endocrinol*. 2008 Aug;159(2):161–5.
- Carlé A, Pedersen IB, Knudsen N, Perrild H, Ovesen L, Rasmussen LB, et al. Development of autoimmune overt hypothyroidism is highly associated with live births and induced abortions but only in premenopausal women. *J Clin Endocrinol Metab*. 2014 Jun;99(6):2241–9.
- Laurberg P, Jørgensen T, Perrild H, Ovesen L, Knudsen N, Pedersen IB, et al. The Danish investigation on iodine intake and thyroid disease, DanThyr: status and perspectives. *Eur J Endocrinol*. 2006 Aug;155(2):219–28.
- Rasmussen LB, Ovesen L, Bülow I, Jørgensen T, Knudsen N, Laurberg P, et al. Dietary iodine intake and urinary iodine excretion in a Danish population: effect of geography, supplements and food choice. *Br J Nutr*. 2002 Jan;87(1):61–9.
- Pedersen IB, Laurberg P, Arnfred T, Knudsen N, Jørgensen T, Perrild H, et al. Surveyance of disease frequency in a population by linkage to diagnostic laboratory databases. A system for monitoring the incidences of hyper- and hypothyroidism as part of the Danish iodine supplementation program. *Comput Methods Programs Biomed*. 2002 Mar;67(3):209–16.
- Carlé A, Laurberg P, Knudsen N, Perrild H, Ovesen L, Rasmussen LB, et al. Thyroid peroxidase and thyroglobulin auto-antibodies in patients with newly diagnosed overt hypothyroidism. *Autoimmunity*. 2006 Sep;39(6):497–503.
- Pedersen CB. The Danish Civil Registration System. *Scand J Public Health*. 2011 Jul;39(7 Suppl):22–5.
- Knudsen N, Bülow I, Jørgensen T, Laurberg P, Ovesen L, Perrild H. Comparative study of thyroid function and types of thyroid dysfunction in two areas in Denmark with slightly different iodine status. *Eur J Endocrinol*. 2000 Oct;143(4):485–91.
- Pedersen IB, Knudsen N, Jørgensen T, Perrild H, Ovesen L, Laurberg P. Thyroid peroxidase and thyroglobulin autoantibodies in a large survey of populations with mild and moderate iodine deficiency. *Clin Endocrinol (Oxf)*. 2003 Jan;58(1):36–42.
- Carlé A, Laurberg P, Pedersen IB, Knudsen N, Perrild H, Ovesen L, et al. Epidemiology of subtypes of hypothyroidism in Denmark. *Eur J Endocrinol*. 2006 Jan;154(1):21–8.
- Chen J, Shao J. Nearest neighbor imputation for survey data. *J Off Stat*. 2000;16:113–31.
- Strieder TG, Prummel MF, Tijssen JG, Endert E, Wiersinga WM. Risk factors for and prevalence of thyroid disorders in a cross-sectional study among healthy female relatives of patients with autoimmune thyroid disease. *Clin Endocrinol (Oxf)*. 2003 Sep;59(3):396–401.
- Benhaim Rochester D, Davies TF. Increased risk of Graves' disease after pregnancy. *Thyroid*. 2005 Nov;15(11):1287–90.
- Effraimidis G, Strieder TG, Tijssen JG, Wiersinga WM. Natural history of the transition from euthyroidism to overt autoimmune hypo- or hyperthyroidism: a prospective study. *Eur J Endocrinol*. 2011 Jan;164(1):107–13.
- Rogers MA, Levine DA, Blumberg N, Fisher GG, Kabeto M, Langa KM. Antigenic challenge in the etiology of autoimmune disease in women. *J Autoimmun*. 2012 May;38(2-3):J97–102.
- Lee HJ, Li CW, Hammerstad SS, Stefan M, Tomer Y. Immunogenetics of autoimmune thyroid diseases: A comprehensive review. *J Autoimmun*. 2015 Nov;64:82–90.
- Srivatsa B, Srivatsa S, Johnson KL, Samura O, Lee SL, Bianchi DW. Microchimerism of presumed fetal origin in thyroid specimens from women: a case-control study. *Lancet*. 2001 Dec;358(9298):2034–8.
- Thomas MR, Williamson R, Craft I, Yazdani N, Rodeck CH. Y chromosome sequence DNA amplified from peripheral blood of women in early pregnancy. *Lancet*. 1994 Feb;343(8894):413–4.
- Evans PC, Lambert N, Maloney S, Furst DE, Moore JM, Nelson JL. Long-term fetal microchimerism in peripheral blood mononuclear cell subsets in healthy women and women with scleroderma. *Blood*. 1999 Mar;93(6):2033–7.
- Watanabe M, Iwatani Y, Kaneda T, Hidaka Y, Mitsuda N, Morimoto Y, et al. Changes in T, B, and NK lymphocyte subsets during and after normal pregnancy. *Am J Reprod Immunol*. 1997 May;37(5):368–77.
- Amino N, Tada H, Hidaka Y. Postpartum autoimmune thyroid syndrome: a model of aggravation of autoimmune disease. *Thyroid*. 1999 Jul;9(7):705–13.
- Carlé A, Pedersen IB, Perrild H, Ovesen L, Jørgensen T, Laurberg P. High age predicts low referral of hyperthyroid patients to specialized hospital departments: evidence for referral bias. *Thyroid*. 2013 Dec;23(12):1518–24.
- Hill AB. The environment and disease: association or causation? *Proc R Soc Med*. 1965 May;58(5):295–300.