

Survey on a Cohort of Subjects Aged From 6 Months to 18 Years and Born to Mothers Managed for Thyroid Hypo-Function in Pregnancy. Experience in a Single Non-Academic Centre in Liguria, Italy

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ABSTRACT

Background: The adequate management of thyroid dysfunction in pregnancy reduces pre-partum adverse events and improves the life of the new-born. **Aim:** Women managed for thyroid hypo-function during pregnancy were retrospectively identified and a survey was performed on their offspring aged from 6 months to 18 years. **Methods:** We selected 131 pregnancies for survey. From January to August 2022, the mothers were sent a questionnaire containing questions on the neonatal and post-natal period and questions on their children's current auxological, scholastic and pathological status and lifestyle. Eighty-two questionnaires were returned. The mothers' replies were compared with their f-T4 and TSH levels recorded during pregnancy. Reference f-T4 and TSH values were extrapolated from 76 pregnancies managed according to current guidelines. **Results:** f-T4 values below the 2.5th percentile were found in less than 3% of cases, while f-T4 values above the 97.5th percentile were found in less than 4%. TSH proved more variable. Replies regarding the time of the new-born's first postural changes were significantly inversely correlated with f-T4 concentrations in each trimester of pregnancy and positively correlated with the TSH levels observed in the 1st trimester. **Conclusions:** This single-centre, cross-sectional, study offers further indications for the management of thyroid hypofunction in pregnancy and suggests that the control of maternal f-T4 and TSH levels during pregnancy has improved in our district. Data from survey indicate that f-T4 influences early motor activity in new-borns more than TSH does. However, the small sample size, the wide age range of the children and the long time-lag between delivery and survey administration could impair our results.

Keywords: Thyroid function, Pregnancy outcome, Offspring outcome, post-natal survey.

BACKGROUND

Although the utility of universal screening of thyroid function in pre-pregnancy is still debated, the increase in age on pregnancy and the growing diffusion of reproductive technologies have increased the need for thyroid examinations in women who wish to become pregnant [1, 2]. Several guidelines have been published to guide decisions to prevent thyroid dysfunction during pregnancy [3-5].

Hypothyroidism, more than hyperthyroidism, is frequently observed in women of reproductive age, and the treatment of overt disease before and during pregnancy is mandatory. However, how subclinical thyroid diseases should be treated is still debated. Subclinical hypothyroidism (SCH) and isolated hypothyroxinaemia (decreased f-T4 levels with normal TSH levels) are the most frequent thyroid dysfunctions encountered in pregnancy [6]. On analysing 47,045 pregnant women, 3.1% of whom had SCH, 2.2% isolated hypothyroxinaemia and 7.5% positive thyroid peroxidase antibody (TPOAb) titres, Korevaar et al. [7] observed a higher risk of pre-term birth. Ge et al. [8] found an increased risk of neuro-developmental disorders in the offspring of SCH mothers with inadequate control of hypothyroidism (TSH levels >4 mIU/l) and underlined the need for routine measurement and timely treatment of thyroid hypofunction in pregnancy. Similarly, Maraka et al. [9] confirmed that SCH in pregnancy was associated with multiple maternal and neonatal adverse outcomes, but failed to ascertain the role of levo-thyroxine (L-T4) therapy in preventing these outcomes. On the other hand, some researchers have reported that L-T4 treatment in SCH pregnant women could improve maternal and neonatal adverse outcomes [10, 11], but many controversial data have emerged on the possible detrimental effects (increased risk of pre-term delivery, small for gestational age neonates, pre-term delivery, gestational diabetes and pre-eclampsia) of potential L-T4 over-treatment [8, 12, 13].

The TSH values above which L-T4 treatment should be started are indicated in the 2017 American Thyroid Association (ATA) guidelines and population- and trimester (Tr)- specific cut-offs are recommended for monitoring therapy [3]. An overview of published gestational TSH and f-T4 reference intervals has recently been published [14]. Reference intervals for TSH and f-T4 in pregnancy vary widely, owing to differences in assay methods, inclusion/exclusion of antibody-positive women or women with thyroid diseases in pregnancy, iodine status, body mass index (BMI) and other additional criteria, and the percentile range used [14-16]. In addition, extrapolating published reference intervals to local reality is quite difficult, and over- or under-treatment in subclinical thyroid dysfunction is somewhat a risk. In a retrospective (1999-2019) analysis of the real-world management of pregnant hypothyroid women in a secondary endocrine centre in our region (Savona district, Liguria, Italy), we observed that adherence to guidelines improved over time, and L-T4 over-treatment decreased [17]. However, several concerns remained, such as infrequent pre-gestational endocrine examinations, referral to the endocrinologist for out-of-range TSH levels late in pregnancy and scant perception of the need for iodine supplementation [17]. Moreover, no significant differences in adverse obstetric or perinatal outcomes were noted in medical records collected over a long period of time [17]. The aim of the present study was to extend previous observations in women attending the same non-academic secondary centre from the pre-gestational period to delivery for the management of thyroid dysfunction, mainly SCH, by providing data on post-natal outcomes, as self-reported by mothers. A secondary aim was to quantify trimester-specific f-T4 and TSH intervals and compare post-natal outcomes with individual hormonal values in pregnancy.

MATERIAL AND METHODS

Subjects

This cross-sectional study was conducted at the Endocrine Unit of Priamar Clinical Diagnostic Centre, a private secondary-level out-patient centre located in the Savona district (Liguria, Italy). From medical files collected from 2000-2022, we identified women of childbearing age

who were treated for thyroid dysfunction; 151 had been managed for 193 pregnancies. They were referred to the centre before or during pregnancy by general practitioners or gynaecologists for the evaluation and management of thyroid dysfunction. Initial exclusion criteria were: mother's age less than 18 years; twin or higher-order gestations; except for L-T4, administration of other medications known to affect thyroid function during pregnancy. Figure 1 details the pregnancies excluded for various reasons and when offspring were aged less than 6 months or over 18 years. After these exclusions, 131 questionnaires were sent to the known email addresses, from January to August 2022. This period was chosen so that the mothers' judgements of scholastic performance could be referred certainly to the last school year, after the schools had closed for holidays. Finally, 82 questionnaires were completed and returned (Figure 1).

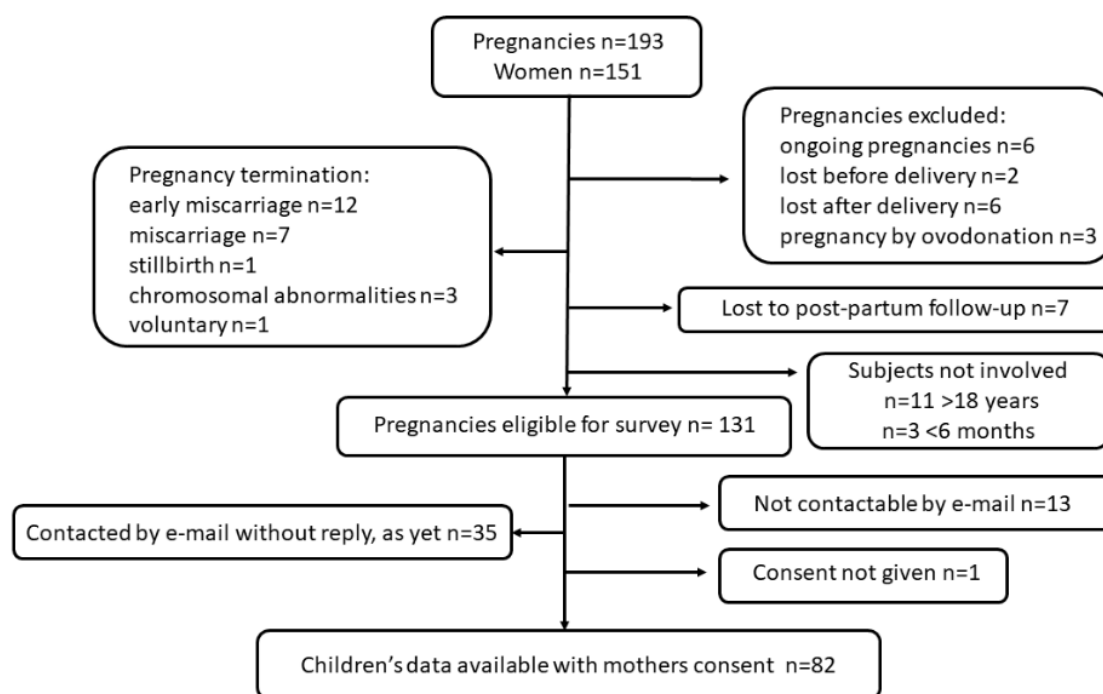


Figure 1 Flowchart of the study.

In these women, the average age at the time of pregnancy was 35.3 ± 4.4 years ($\pm$ SD; range 25-46 years). Thyroid diagnoses were: Hashimoto's thyroiditis (HT; n=54), uni-nodular or multi-nodular goitre (n=14), thyroid ultrasonography (US) indicative of HT but with negative thyroid antibodies (n=10), and post-surgical hypothyroidism (n=4). In 70% of pregnancies, a pre-gestational examination was available; in 77% of pregnancies, L-T4 treatment was ongoing after the finding of a positive β -human chorionic gonadotropin value. Thyroid function was managed in accordance with the available literature data [3, 18, 19] and as previously reported [17]. In general, during the pre-pregnancy examination, women were informed of the need to start L-T4 or to increase their L-T4 dosage when pregnancy began. After the first adjustment, L-T4 was increased arbitrarily by 25 μ g/week per 2 kg gain in body weight during pregnancy [17]. An iodine intake of >150 μ g/day was recommended during the pre-gestational visit and/or at the first examination during pregnancy. Prenatal endocrine examination was performed in the 1st trimester (median 8 weeks; IQR 6-10 weeks, range 4-12 weeks) in the

majority (90%) of pregnancies, while in the remaining cases it was performed later (median 16 weeks, IQR 14-19 weeks, range 13-24 weeks). In all pregnancies involved in the survey, body mass index (BMI), pre-pregnancy smoking, parity, numbers of subjects with TPOAb positive titres, drugs taken during pregnancy, gestational week of delivery, percentages of women with pre-term delivery, birth weight and APGAR score [20] were reported (Table 1). As a reference, the corresponding values were extrapolated from a group of 69 women with 76 pregnancies (Table 1). In the majority of these pregnancies (91%) thyroid dysfunction during pregnancy was managed according to the 2017 ATA guidelines [3]. Thyroid diagnoses were: HT (n=42), uni-nodular or multi-nodular goitre (n=11), US indicative of HT but with negative thyroid antibodies (n=11), and post-surgical hypothyroidism (n=5). In the remaining 7 women, thyroid function was considered normal and no L-T4 treatment was started.

Table 1 Some clinical variables recorded on examination during pregnancy and data on the outcome of pregnancy in women involved in the survey. Data provided by survey respondents were compared with those recorded in a group of reference women (see "Subjects" for details).

Variable	Women involved in the survey	Reference women	Significance P
Number of women	65	69	
Number of pregnancies	82	76	
Age (years; mean $\pm$ SD)	35.3 $\pm$ 4.4	35.4 $\pm$ 4.5	0.81
BMI (median; IQR)	22.0 (19.9 – 24.3)	21.9 (19.8 – 24.6)	0.90
Pre-pregnancy smokers (%)	53	24	0.003
Previous birth (%)	34	34	0.99
Week on 1 st thyroid analysis (median; IQR)	8 (6 – 10)	8 (6 - 9)	0.28
TPOAb positivity (%)	62	59	0.63
on L-T4 therapy (%)	77	56	0.02
Other drugs ongoing (n)			
None	5	4	0.99
Foline	74	67	0.80
Iodine	24	31	0.09
Iron	5	3	0.72
Other vitamins	4	6	0.52
Other drugs	15	10	0.39
Gestational delivery (weeks; median; IQR)	38 (37 – 40)	39 (37 – 40)	0.74
Pre-term delivery ($\leq$ 36 weeks; %)	17	12	0.67
Caesarean section (%)	32	48	0.06
Birth weight (kg; median; IQR)	3.19 (2.84 – 3.45)	3.17 (2.75 – 3.58)	0.88
Apgar score (1 min; median; IQR)	9 (9 – 9)	9 (9 – 9)	0.36
Apgar score (5 min; median; IQR)	10 (10 – 10)	10 (10 – 10)	0.59

Protocol

Several pregnancies were lost or withdrawn. Owing to the length of time from delivery to the survey, 10 women (n=13 pregnancies) were not contactable, as their previous e-mail addresses were no longer valid. At the time of data entry, 28 women (n=35 pregnancies) had not yet replied. Only 1 woman withheld consent (Figure 1).

During pregnancy, endocrine surveillance was conducted by means of e-mails containing clinical data (estimated week of gestation, body weight, arterial pressure, L-T4 dosage, changes in other therapies), thyroidal data (TSH, f-T4) and some clinical biochemistry data (full blood count, glycaemia and urinalysis). Data (f-T4, TSH, week of pregnancy) received in the same trimester were collected and expressed as mean values. At the end of gestation, mothers were followed up as previously reported [17]. During post-pregnancy examination, data on delivery and the neonate at birth were also collected. The questionnaire was written in Italian and sent to eligible women, who were asked to provide some data on physiological changes in the first months of their baby's life. At the time of data collection, auxological, nutritional and, when applicable, scholastic parameters and children's aptitudes were also recorded. The questionnaires concerned children aged >6 months and <18 years at the time of the survey.

Outcomes

The primary aim was to correlate the child's post-natal physiological changes, subsequent auxological parameters and aptitudes with the mother's thyroid function during pregnancy. The secondary aim was to correlate delivery outcomes with thyroid function during pregnancy. An additional outcome was to compare gestational data on women involved in the survey with data from a reference group of pregnant women followed up after publication of the latest guidelines on thyroid management during pregnancy [3].

Methods

We defined trimesters as 0-12 weeks (1st Tr), 13-26 weeks (2nd Tr) and ≥ 27 week (3rd Tr) of gestation. The BMI of the women was calculated on the basis of the weight (kg) and height (m) reported in medical files, according to the following formula: kg/m^2 . Marked gestational weight gain (GWG) was defined according to Institute of Medicine guidelines [21]. Smoking was stratified as yes/no; women who stopped smoking during pregnancy were regarded as smokers. We based the diagnosis of HT on the documentation of TPOAb positivity and/or a heterogeneous hypoechoic pattern on US examination according to Rago et al. [22]. SCH was defined as a serum TSH level from 4 to 10 mIU/L with normal serum f-T4. Overt hypothyroidism (OH) was defined as a TSH level >4 mIU/l with decreased f-T4 concentration, or as TSH >10 mIU/L regardless of the f-T4 level. Definitions were taken from the 2017 ATA recommendations [3]. Gestational hypertension was defined as new-onset hypertension without proteinuria, with blood pressure $\geq 140/90$ mmHg after 20 weeks' gestation [23]. Gestational diabetes mellitus (GDM) was defined according to the standard diagnostic criteria established by the American Diabetes Association [24]. Pre-term delivery was defined as birth before the 37th week of gestation. Neonatal admission was defined as the admission of a neonate to the neonatal intensive care unit (NICU). The anthropometric parameters of weight and height recorded in children were analysed by means of an online calculation program (www.childmetrics.org) [25] and transformed into age- and sex-specific percentiles, according to the reference cards of the Centers for Disease Control and Prevention (www.cdc.gov/growthcharts/clinical_charts.htm). A score was assigned to the mother's

judgement of her child's physical activity (0=none, 1=yes, 2=competitive sport) and scholastic results (1=adequate, 2=good, 3=very good).

Laboratory Examinations

Serum samples were analysed in the laboratory of the Public Health Service of Azienda Sanitaria Ligure 2 in Savona (Italy) by means of electrochemiluminescence immunoassay, using the Cobas automated analytic platform (Roche Diagnostics, Mannheim, Germany). Normal non-pregnancy ranges are 9.3 to 17.1 pg/ml for f-T4 (to transform pg/ml to pmol/l, divide by 0.777) and 0.3 to 4.2 mIU/l for TSH. The functional sensitivity of the TSH assay is 0.03 mIU/l, with intra- and inter-assay coefficients of variation of 3% and 7%, respectively. No Tr-specific f-T4 and TSH ranges are available. From 2017 onwards, normal TSH reference values were taken from the available literature [3, 25]. Several commercial methods were used for TPOAb evaluation during the study period, and judgements of negative TPOAb values were assigned according to the normal range reported by the manufacturers.

Statistical Analysis

GraphPad 9.5.1 software (GraphPad, San Diego, CA, USA) was used for statistical analysis. Absence of normality was tested by means of the Kolmogorov-Smirnov test. To compare continuous data, the Mann-Whitney test was used. To analyse Tr changes after a significant Kruskal-Wallis test, we used Dunn's multiple comparison test. Percentages were compared by means of Fisher's exact test. Correlations were evaluated by means of univariate (Spearman test) and multivariate (Least squares) correlations. Categorical variables were described as percentages, and continuous variables as mean $\pm$ standard deviation (SD), median, and inter-quartile ranges (IQR). Significance was set at $P \leq 0.05$

Ethical Approval

All patients had provided written informed consent the management of data collected in their medical files before their examination at Priamar Clinical Diagnostic Centre, and had agreed to their use for scientific purposes. In addition, the returned questionnaires were analysed only if a new written informed consent form was signed by the mothers. Owing to the retrospective nature of collection of clinical and hormonal data on the women and their children, no further formal approval from the Liguria Ethics Committee was required. Data were managed anonymously. Data collection and subsequent analysis were performed in compliance with the Helsinki Declaration.

RESULTS

Pre-Delivery and Delivery Data

The characteristics of the 65 women involved in the survey and the outcomes of their 82 pregnancies are reported in table 1. A significantly higher percentage of smokers was observed in these women (53%) than in the group of reference women (24%, $P=0.003$). At the first endocrinological examination, L-T4 therapy was ongoing in 77% of pregnancies. This percentage was significantly greater than that observed in the reference pregnancies (56%; $P=0.02$). At the first examination, iodine supplementation had already been started in only 29% of pregnancies, a slightly lower percentage than that reported in the reference pregnancies (41%). The median gestational delivery, the percentage of pre-term delivery, birth weight and APGAR scores did not differ between the survey group and the reference group. Caesarean sections were more frequent in the reference group than in the survey group (48% vs. 32%;

P=0.06) (Table 1). New-onset hypertension needing hypotensive treatment was only registered during 2 pregnancies in the survey group and during 1 pregnancy in the reference group. GDM was observed in 2 and 3 pregnancies in the survey group and the reference group, respectively. GWG was evaluable in 59 pregnancies in the survey group and in 46 in the reference group, and was observed in 25% and 20%, respectively (P=0.13). Admission to the NICU due to respiratory distress was registered in 1 and 3 newborns in the survey group and references group, respectively.

Figure 2 shows Tr-specific values of f-T4 and TSH in the women involved in the survey. A significant decrease in f-T4 levels was observed both in women involved in the survey (Kruskal-Wallis test; $P < 0.0001$) and in reference women ($P = 0.0001$), while TSH changes were more variable in the survey women ($P = 0.003$) than in the reference women (Figure 2).

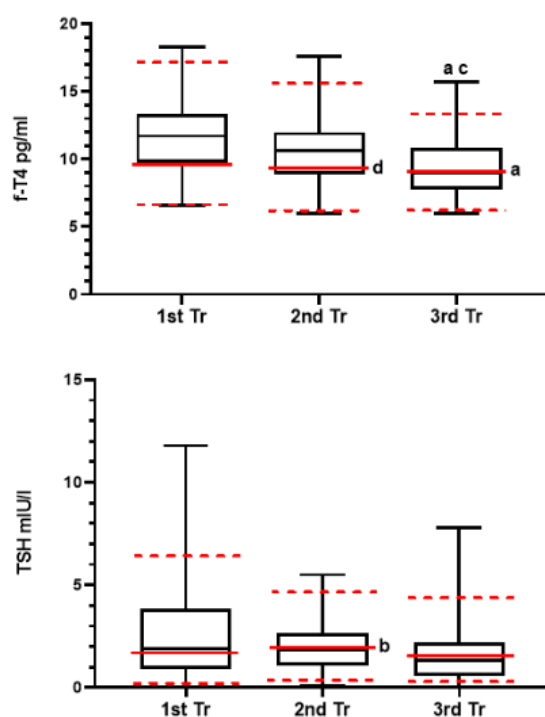


Figure 2 Median (IQR and range) of f-T4 and TSH values in each trimester recorded in the survey participants. Solid red lines indicate the median values while dashed red lines indicate the 2.5th and 97.5th percentiles of f-T4 and TSH values recorded in pregnant women in the reference group. Significance: a) $P = 0.0001$ vs 1st Tr; b) $P = 0.002$ vs 2nd Tr; c) $P = 0.01$ vs 2nd Tr; d) $P = 0.01$ vs 1st Tr.

In women involved in the survey, f-T4 values below the 2.5th percentile (reference women: 1st Tr 6.6 pg/ml, 2nd Tr 6.0 pg/ml, 3rd Tr 6.2 pg/ml) were found in 1% during the 1st Tr and in 3% from the 2nd to 3rd Tr, while f-T4 values above the 97.5th percentile (reference women: 1st Tr 17.2 pg/ml, 2nd Tr 16.8 pg/ml, 3rd Tr 13.2 pg/ml) were found in 2%, 4% and 3% from the 1st to 3rd Tr. In survey women, TSH values below the 2.5th percentile (reference: 1st Tr 0.05 mIU/l, 2nd–3rd Tr 0.17 mIU/l) were found in 0%, 3% and 7% from the 1st to 3rd Tr, while TSH values above the 97.5th percentile (1st Tr 6.42 mIU/l, 2nd Tr 4.71 mIU/l, 3rd Tr 4.41 mIU/l) were found

in 7%, 1% and 3% from the 1st to 3rd Tr. Women involved in the survey displayed a significant ($P=0.02$) decreasing trend in TSH levels above the 97.5th percentile from the 1th Tr to the 3rd Tr, with a non-significant ($P=0.06$) increase in TSH levels <2.5th percentile. The percentage changes in f-T4 levels below the 2.5th ($P=0.6$) or above the 97.5th ($P=0.8$) percentiles were not significant.

Post-Delivery and Childhood

When the questionnaires were collected, the average age of the children was 6.9 ± 4.5 years (median, IQR: 6.3 years, 3.0-9.2 years; range 0.6-16.2 years). Forty-five (55%) questionnaires were fully compiled by the mothers. In 14 (17%) questionnaires, some answers regarding the post-natal period were incomplete, owing to the time-lag between birth and data collection (e.g., mother's reply: "I don't remember"). In 23 (28%) questionnaires, some replies were missing ($n=6$) owing to the non-applicability of the answer due to the young age of the child (e.g., autonomous walking) and/or ($n=19$) pre-school age (e.g., physical activity, scholastic performance). No completely blank questionnaire was returned. Six children were taking anti-allergic drugs, alone or in combination (anti-histamine $n=5$; β 2-stimulating $n=1$, steroids $n=1$), and 3 were taking cholecalciferol. One 5.7-year-old boy was taking flecainide for arrhythmia and a 12.0-year-old girl was on L-T4 for acquired HT with SCH. Two children were below the 5th percentile in height, one of whom, a girl aged 8.7 years, with neuroradiological finding of empty sella, was under evaluation for childhood GH deficiency; a 6.2-year-old boy with a history of liver transplantation for autoimmune liver disease was taking several drugs against host-rejection. Three boys (5.1, 6.4, 11.8 years old) were under neuropsychiatric examination for global delay in psychomotor development, *attention deficit hyperactivity disorder*, and high-functioning autism spectrum disorder, respectively.

Table 2 reports the results of the questionnaires returned by the women involved in the survey. Language development was delayed in a 6.4-year-old child with *attention deficit hyperactivity disorder*. Two sisters, aged 9.3 and 12.0 years, were below the 5th percentile in weight, but no signs of pre-pubertal anorexia were reported; their parents had a similar slim phenotype. In addition, a 9.0-year-old girl, who practised ballet dancing for 4 hours/week, and an 8.1-year-old boy were below the 5th weight percentile. Weight above the 95th percentile was reported only in a 4.8-year-old female and an 8.2-year-old male. No sports were reported in 14 questionnaires (20%) in which this item was evaluable; the most frequently reported sports were water sports ($n=12$; 18%), probably because Savona is situated on the coast and has high-level water polo and synchronized swimming teams. A large number of different hobbies were reported (Table 2).

Table 2 Analysis (median, IQR, range) of replies to the questionnaire returned by the women involved in the survey. Legend: 1) other diets comprise: n=9 restricted owing to food intolerances, n=5 scheduled weaned, n=1 vegetarian; 2) physical activity outside school: 0 = none; 1 = amateur, on average >120 minutes per week; 3 = competitive; 3) mother's judgement of scholastic performance: 1 = adequate, 2 = good, 3 = excellent. 4) Children of school age with non-sporting hobbies: NA = not applicable owing to age.

		Median	IQR	Range	Questionnaires evaluable (n)
Post-natal data					
Exclusive breastfeeding length	months	5.0	1.0 – 9.3	0.0 – 24.0	82
Starting postural changes	months	6.0	5.0 – 7.0	3.0 – 9.0	73
Starting autonomous walking	months	12.0	12.0 – 14.0	9.0 – 18.0	75
Starting babbling	months	6.5	4.0 – 10.5	3.5 – 21.5	74
First teeth eruption	months	7.0	6.0 – 9.0	3.0 – 14.0	71
Current data					
Age when questionnaire was returned	years	6.3	3.0 – 9.2	0.6 – 16.2	82
Weight	Percentile	57.9	29.6 – 76.8	1.8 -97.1	82
Height	Percentile	51.0	22.9 - 74.9	1.0 - 99.6	82
Diet mixed/other diets (1)	Subjects	67/15	-	-	82
Physical activity (2)	Score	1	1 - 1	0 - 2	68
School report (3)	Score	2	2 - 3	1 - 3	53
Subjects with hobbies (4)	Yes/No/NA	36/20/24	-	-	80

Tr-specific f-T4 and TSH values recorded in the mothers were correlated with the data obtained from the questionnaires. Table 3 reports the univariate correlation analysis. Only the replies to the item regarding the time of the first postural changes were significantly inversely related to the f-T4 concentrations observed in each Tr of pregnancy (Figure 3).

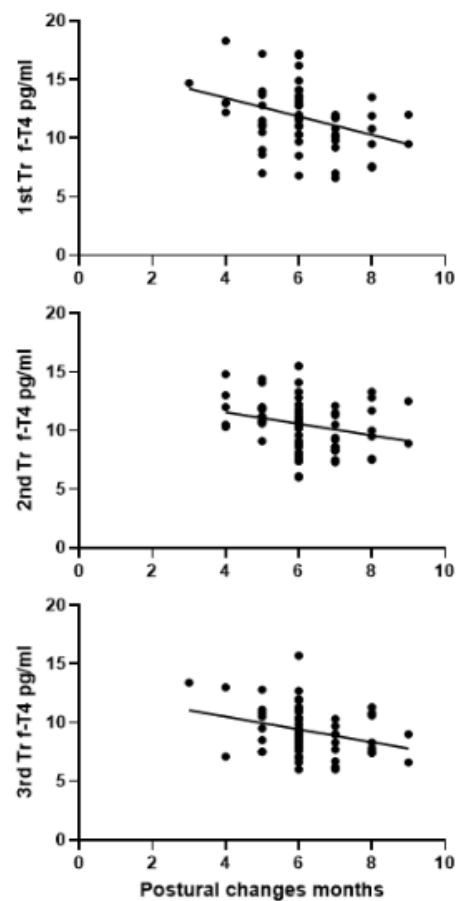


Figure 3 Correlation between early postural changes and f-T4 values recorded in each trimester of pregnancy (see also table 3).

This item was positively correlated with the TSH levels observed in the 1st Tr of pregnancy (Table 3). In addition, a sporadic inverse correlation was noted between 2nd Tr f-T4 values and both the eruption of the first teeth and months of breastfeeding, and between 1st Tr TSH values and the current weight percentile in children (Table 3). On multivariate analysis, the item “first postural changes” remained significantly correlated with the dependent variable f-T4 only in the 1st Tr (P=0.03).

Table 3 Univariate correlation between the women's replies to the items in the questionnaire and the f-T4 and TSH values recorded during each trimester of pregnancy.

	1 st Tr			2 nd Tr			3 rd Tr		
F-T4 correlation	Pair (n)	Sr	Significance, P	Pair (n)	Sr	Significance, P	Pair (n)	Sr	Significance, P
Exclusive breastfeeding	51	-0.17	0.23	57	-0.40	0.002	53	-0.22	0.12
Starting postural changes	58	-0.36	0.006	65	-0.29	0.02	58	-0.28	0.04
Starting autonomous walking	61	-0.09	0.47	66	-0.14	0.28	58	0.14	0.28
Starting babbling	62	0.06	0.66	67	-0.14	0.26	60	-0.12	0.36
First teeth eruption	58	-0.24	0.06	63	-0.25	0.05	56	-0.00	0.98
Current weight percentile	65	0.18	0.15	71	0.05	0.68	65	0.15	0.22
Current height percentile	65	0.08	0.52	71	0.01	0.96	65	0.11	0.37
Current physical activity	54	0.01	0.93	59	0.09	0.48	52	0.22	0.12
Scholastic report	44	-0.01	0.93	44	-0.23	0.13	38	0.00	0.99
TSH correlation	Pair (n)	Sr	Significance P	Pair (n)	Sr	Significance P	Pair (n)	Sr	Significance P
Exclusive breastfeeding	54	0.13	0.35	63	0.08	0.54	57	0.14	0.30
Starting postural changes	60	0.31	0.01	69	0.17	0.15	64	0.20	0.11
Starting autonomous walking	62	0.19	0.14	70	0.05	0.68	64	-0.02	0.87
Starting babbling	63	-0.14	0.26	70	-0.22	0.07	66	-0.05	0.70
First teeth eruption	59	0.23	0.09	68	0.03	0.79	61	-0.23	0.06
Current weight percentile	67	-0.27	0.03	77	-0.14	0.23	71	-0.17	0.16
Current height percentile	67	-0.07	0.55	77	-0.12	0.28	71	-0.07	0.09
Current physical activity	54	0.01	0.94	63	-0.02	0.82	57	-0.17	0.21
Scholastic report	43	0.08	0.62	48	-0.01	0.95	43	0.06	0.70

DISCUSSION

The evaluation of thyroid function in women of childbearing age and the management of thyroid dysfunction in pregnancy and the post-partum period are topical issues [4, 17]. To date, several increased risks in SCH have been reported in systemic reviews and meta-analyses [6-10, 27, 28]. The degree of positive thyroid autoimmunity and SCH appears to have a synergic effect in increasing the risk of adverse obstetric outcomes [29]. Studies of isolated hypothyroxinaemia, however, have yielded mixed results [6, 27]. The association between mild maternal thyroid hypofunction and child development seems to indicate that maternal f-T4 levels, rather than maternal TSH, are predictive of a child's neurodevelopment [30]. However, identifying abnormalities in thyroid function tests during pregnancy may be complicated by physiological maternal changes, the absence of reference intervals, population characteristics and the assays used [14].

Moreover, the recommended use of population- and Tr-specific TSH and f-T4 reference values is often not feasible [3]. In the present study, local Tr-specific TSH and f-T4 reference intervals were not available, which could constitute a limitation. On the other hand, the lower range of f-T4 in the women involved in our survey (1st Tr 6.6 pg/ml, 2nd-3rd Tr 6.0 pg/ml) was similar to that reported by Osinga et al. [14] in 63,198 pregnant women (6.1 pg/ml, 5.5 pg/ml, and 7.4 pg/ml from 1st to 3rd Tr); the upper limits of f-T4 levels in our study (18.3 pg/ml, 17.6 pg/ml and 15.7 pg/ml from 1st to 3rd Tr) were also very similar (18.5 pg/ml, 17.6 pg/ml and 15.2 pg/ml from 1st to 3rd Tr) to those reported by these authors [14]. However, TSH displayed greater variability than that reported by Osinga et al. [14], indicating that in some of our women SCH was under- or over-treated. Moreover, when we arbitrarily compared the f-T4 and TSH data from women involved in the survey with those obtained from the reference women followed up after the 2017 release of the latest ATA guidelines [3], we observed that stricter adherence to guidelines [3] and to our *ad hoc* protocol of L-T4 increase and monitoring in pregnancy [17] reduced f-T4 and TSH variability and SCH over-treatment (Figure 2).

We observed that 30% of the women in our survey group were referred for endocrine evaluation when still pregnant, sometimes after the 1st Tr of pregnancy (10%). Recently, Moleti et al. [31] reported optimal preconception TSH cut-off levels in SCH women. In women recruited from 2008 to 2017, these authors observed that TSH cut-offs of 1.2 mIU/l and 1.7 mIU/l seemed to guarantee 1st Tr TSH values below 2.5 mIU/l and below 4.0 mIU/l, respectively. Comparison between our study and that of Moleti et al. [31] probably reflects a difference in the length of the data collection period (ours was 2000-2022) and some differences in access to treatment (public vs private).

Guidelines [3, 32], trials [33] and spontaneous studies [17] indicate that L-T4 therapy must be increased by approximately 20-30% in SCH women with a suspected or ascertained pregnancy. In our experience, after the first adjustment of L-T4 dosage, progressively increasing the L-T4 dosage according to the weight increase (arbitrarily 25 µg/weekly every 2 kg gained) reduces the risk of SCH progression. In our pregnancies, after the late 2nd Tr, low f-T4 levels and/or high TSH levels were observed in only about 3% of women, while the risk of moderate hyperthyroxinaemia was much higher: 3-7%. In the *post hoc* analysis of the TABLET trial, in which TPOAb-positive euthyroid women were assigned to receive either 50 µg L-T4 or placebo, Gill et al. [34] reported a higher risk of developing hypothyroidism in untreated women (9%) than in L-T4 treated women (6%), while thyrotoxicosis developed in 4% of women in this latter

group. These and our data indicate that dynamic monitoring of L-T4 treatment must also be continued during pregnancy.

Several other factors, including behavioural, nutritional and metabolic factors, are involved in the outcome of pregnancy in SCH women. In our study, pre-pregnancy smoking was more frequent in the survey women than in the reference women. By contrast, at the beginning of pregnancy, iodine supplementation was more frequent in the reference group than in the survey group. It is well known that the iodine requirement increases in pregnancy [35] and that an inadequate iodine intake can impair the child's postnatal intellectual development [36]. Our data show that, from 2000 to 2022, healthier behaviours (reduced pre-pregnancy smoking; iodine integration early in pregnancy) seem to have been effective in the Savona district. We cannot affirm that, in the group of women involved in our survey, a low iodine intake during pre-pregnancy, pregnancy and lactation might have caused some subsequent harm to their children. However, since 2017 gynaecologists and endocrinologists in our district have increased their effort to improve iodine intake in women of childbearing age. The benefits of this improvement could be manifested in the future. By contrast, Moleti et al. [31] reported that only 70% of 260 women were on iodine supplementation at the beginning of pregnancy; this observation, like that reported by us in patients with thyroid disease [37], should prompt reconsideration of the data from the last Italian ministerial survey [38].

Optimal control of thyroid function in pregnancy is necessary, as women with low f-T4 levels are reported to have a greater risk of hypertension disorder in pregnancy [39]. In our study, only two women (one in each group) presented gestational hypertension, without isolated hypothyroxinaemia, at the end of pregnancy (1.2% of pregnancies). Su et al. [39] have suggested that only persistent hypothyroxinaemia (f-T4 level <10th percentile; TSH within normal reference range) is associated with an increased risk of pre-eclampsia/ eclampsia. Finally, the interplay between thyroid control in pregnancy and GWG was evaluated in our study, and GWG was found in about one in four pregnancies evaluable, without differences between the survey group and the reference group. This finding seems to be lower than that reported in a recent meta-analysis, which showed that Institute of Medicine recommendations [21] on GWG were frequently disregarded [40].

Further limitations of our survey were: the low number of questionnaires returned, the self-reported nature of the questionnaires, the wide age range of the children concerned, and the time-lag between delivery and questionnaire administration. Indeed, some questions were not applicable to pre-school age children, and mothers whose children had been born several years earlier were sometimes unable to answer questions about the first months of the new-born's life. In spite of these limitations, however, a significant correlation was found between the f-T4 level in each Tr of gestation and the baby's postural changes. That the beginning of motor activity was associated with maternal f-T4 appears an important finding of our study. Indeed, this observation underlines the role of maternal f-T4 in prenatal neurodevelopment [42, 43] and confirms the need for intensive control of thyroid function and L-T4 supply in mothers with SCH. Moreover, some authors have reported that children born to mothers with hypothyroxinaemia on adequate thyroid hormone replacement therapy display similar development to that of children born to euthyroid mothers [44]. In our study, from 1% to 3% of the women involved in the survey had f-T4 levels below the 2.5th percentile during the pregnancy. Interestingly, Pop et al. [45] compared children born to mothers with f-T4 below

the 10th percentile with children born to mothers with f-T4 percentiles above 50th and found that the former had lower scores on the Bayley motor subscales. Recently, Wang et al. [46] used the same scales to assess the neurodevelopment of infants; they observed that high-normal maternal TSH concentrations were associated with lower gross motor scores only when f-T4 levels were reduced. That finding, and our observation that postural changes were positively related only with the TSH levels observed in the 1st Tr of pregnancy, suggest that greater attention should be paid to decline of f-T4 in pregnant women with TSH at the limit of the normal range in pregnancy. From our data, we could conclude that an adequate L-T4 supply has a favourable effect on early motor activity. Furthermore, the duration of breastfeeding was significantly inversely related to f-T4 concentrations only in the 2nd Tr of pregnancy. This finding needs further evaluation, owing to the well-known benefits of breastfeeding for both the new-born and the mother [47].

The absence of correlations between the other outcomes investigated in the survey and maternal f-T4 and TSH levels could also be due to maternal overestimation of their children's school performance and physical and extra-curricular activities. Literature data on the scholastic performance of children born to mothers with SCH that is not well controlled in pregnancy are limited. Ghassabian et al. [48] found that children born to mothers with f-T4 below the 5th percentile during pregnancy scored an average of 4 points less on intelligence tests at 7 years of age than children of similar age born to euthyroid mothers. Moreover, Pääkkilä et al. [49] reported that maternal thyroid dysfunction in early pregnancy negatively influenced the school performance of 8- and 16-year-old offspring, and that boy, but not girls, had a 5-6-fold higher risk of repeating the school year.

Great attention is focused on impaired neuropsychological development in infancy. In our survey, 3 boys, aged 6-11 years, presented neuropsychological problems (global delay in psychomotor development, attention deficit hyperactivity disorder, and high-functioning autism spectrum disorder). The 3.6% incidence of neuropsychological problems in our survey does not seem to be different from that currently reported [50]. Recently, Su et al. [51] observed that maternal TPOAb positivity was associated with about a two-fold higher risk of neurophysiological problems; however, in only 2 out of 3 mothers of children with neuropsychological problems was thyroid autoimmune disease diagnosed before pregnancy. The pathogenesis of autism spectrum disorders is extremely complex, involving environmental factors, genetic variants, maternal factors and medication use during pregnancy, but no hypothesis seems to involve maternal thyroid levels in pregnancy [50].

In conclusion, our single-centre cross-sectional study offers further indications for the management of thyroid hypofunction in pregnancy and suggests that the control of maternal f-T4 and TSH levels during pregnancy has improved in our district. The data from our survey indicate that f-T4 influences early motor activity in new-borns more than TSH does. However, the small sample size, the wide age range of the children and the long time-lag between delivery and survey administration could impair our results. Further similar research is therefore required.

Abbreviations

f-T4: free-thyroxine; TSH: thyroid-stimulating hormone; SCH: sub-clinical hypothyroidism; American Thyroid Association: ATA; Tr: trimester; Levo-thyroxine: L-T4; TPOAb: thyroid

peroxidase antibodies; BMI: body mass index; HT: Hashimoto's thyroiditis; US: ultrasonography; OH: overt hypothyroidism; GDM: gestational diabetes mellitus; GWG: gestational weight gain; NICU: neonatal intensive care unit; SD: standard deviation; IQR: interquartile range.

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Availability of Data and Materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval and Consent to Participate

All procedures were carried out in accordance with the ethical standards of the institution and with the 1975 Helsinki Declaration, as revised in 2008. Informed consent was obtained from all women.

Competing Interests

No competing financial interests exist.

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