THYROID DISORDERS IN CHILDREN BELOW 3RD YEAR OF LIFE: AGE-RELATED SPECIFICITY AND CHALLENGES

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Table of Contents

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Editorial: Thyroid disorders in children below 3rd year of life: Age-related specificity and challenges

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Editorial on the Research Topic:
Thyroid disorders in children below 3rd year of life: Age-related specificity and challenges

Introduction

Thyroid disorders in children below 3 years old can lead to the deterioration of mental and physical development. This period is critical in the process of activity-dependent synaptogenesis and its plasticity (1) and thyroid hormones play a key role in this process. Furthermore, congenital hypothyroidism is the major cause of intellectual disability which could be completely preventable if diagnosed in time.

The breakthrough in this issue was the introduction of neonatal screening for congenital hypothyroidism established in many countries around the world. Considerable experience regarding diagnostics, treatment, and effects of this rare condition is currently available thanks to the collected data. Based on this experience, numerous articles concerning thyroid disorders have been published, thus improving the care of patients (2, 3). The recently updated guidelines from the European Reference Network on Rare Endocrine Conditions (ENDO-ERN) for congenital hypothyroidism (CH) determine the timing, dosage, and monitoring of the therapy (2). Nevertheless, some particular aspects of the disorder are still not entirely elucidated. A considerable challenge is the assessment of thyroid axes in preterm babies and neonates born by mothers with thyroid disease.

Hypothyroidism before the 3rd year of life

The most common cause of this condition is CH, which in the majority of cases is detected in the neonatal screening test. In children younger than 3 years old, when the brain development is still undergoing its critical phase, a deficiency as well as an excess of thyroid hormones could cause serious consequences. The proper treatment should be well balanced, avoiding undertreatment as well as thyrotoxicosis. The recommended initial dose of levothyroxine (LT4) is 10-15mcg/kg/d for each neonate with a decreased level of fT4 whereas, in children with fT4 within normal ranges, the initial dose could be 5-10mcg/kg/day (2, 3). These doses most likely avoid overtreatment. However, the optimal LT4 dosage is still under debate. In a Dutch study (4), it was found that both early over- and undertreatment may lead to permanent behavioral problems, the former to ADHD and the latter to autism spectrum disorders. It seems that a more precise individualization of dosage, according to the presence of the thyroid gland and initial thyroglobulin level, as well as the potential absorption and bioavailability of LT4 preparations should be considered. Concerning this issue in our Research Topic, Lipska et al. analyze the problems of treatment in 99 children diagnosed with primary CH and describe 5-years worth of experience considering under- and overtreatment. In addition, Esposito et al. analyze the effect of initial levothyroxine dose on neurodevelopmental and growth outcome in a group of children with CH in a prospective randomized trial and Stagi et al. describe the new possibilities of treatment with different LT4 formulations and varying factors influencing the process. Furthermore, Tuli et al. report the primary results of comparison between two liquid LT4 formulations in the treatment of CH.

Another issue is the prevalence of hypothyroidism in preterm newborns. A review of literature on this topic is presented by Kłosińska et al. As the complement to this review, two unrelated analyses based on large groups of preterm babies by Stawerska et al. and Mikołajczak et al. are published. In the latter paper, the authors report the unique data regarding thyroid volume and thyroid axis function in children born before 33 weeks of gestation. In spite of extensive data, there are still unsolved questions. The current recommendations define the indications for diagnostics and LT4 treatment in these children (2, 3, 5), but it is still necessary to update the knowledge in this area.

The thyroid dysfunction before 3 years of life is not always dependent on inborn defects or prematurity. Acquired autoimmune thyroiditis is also reported in very young children (6). Caprio et al. report a case of acquired overt hypothyroidism in a child in their second year of life dependent on iodine deficient hypoallergic diet. This condition should be considered in some children on special diet regardless of sufficient iodination of general population.

Silva et al. report the increased risk of Helicobacter pylori infection observed in children with CH and discuss the possible mechanisms influencing the relationships between these two conditions.

Hyperthyroidism before the 3rd year of life

Hyperthyroidism before the 3rd year of life concerns the neonatal period in the majority of cases and is affiliated with maternal Graves' disease (GD). It could originate from neonates' mothers with active GD but also euthyroid with a history of GD. Severely affected children are at risk of craniosynostosis, cardiac insufficiency, and thyroid associated ophtalmopathy (TAO). The guidelines for the management of this condition define the anti-TSHR antibodies as the most important predictor of neonatal GD (7–9). In our Research Topic, Pyrżak et al. analyze a long follow-up of thyroid function and psychophysical development in children with neonatal hyperthyroidism while Dong et al. reporte the therapeutic perspectives in TAO in pediatric population.

Conclusions

This Research Topic provides an important contribution to the discussion on thyroid disorders in very young children. Although hypo- and hyperthyroidism in children before the 3rd year of life are both rare conditions, they can significantly affect the child's future development and quality of life. Early diagnosis and adequate pharmacological treatment are effective means to resolve the alterations and avoid irreversible consequences of thyroid alterations in this particular age, as documented by the clinical studies presented in this Research Topic. Moreover, the collected papers demonstrate the need for further prospective studies with long-term follow-up concerning the effects on physical, intellectual, and behavioral development in children with early onset thyroid disorders.

Author contributions

AK, MW conceptualized, designed, wrote, and approved the Editorial. All authors contributed to the article and approved the submitted version

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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REVIEW
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Congenital Hypothyroidism in Preterm Newborns – The Challenges of Diagnostics and Treatment: A Review

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Preterm newborns are forced to adapt to harsh extrauterine conditions and endure numerous adversities despite their incomplete growth and maturity. The inadequate thyroid hormones secretion as well as the impaired regulation of hypothalamus-pituitary-thyroid axis may lead to hypothyroxinemia. Two first weeks after birth are pivotal for brain neurons development, synaptogenesis and gliogenesis. The decreased level of thyroxine regardless of cause may lead to delayed mental development. Congenital hypothyroidism (CH) is a disorder highly prevalent in premature neonates and it originates from maternal factors, perinatal and labor complications, genetic abnormalities, thyroid malformations as well as side effects of medications and therapeutic actions. Because of that, the prevention is not fully attainable. CH manifests clinically in a few distinctive forms: primary, permanent or transient, and secondary. Their etiologies and implications bear little resemblance. Therefore, the exact diagnosis and differentiation between the subtypes of CH are crucial in order to plan an effective treatment. Hypothyroxinemia of prematurity indicates dynamic changes in thyroid hormone levels dependent on neonatal postmenstrual age, which directly affects patient's maintenance and wellbeing. The basis of a successful treatment relies on an early and accurate diagnosis. Neonatal screening is a recommended method of detecting CH in preterm newborns. The preferred approach involves testing serum TSH and fT4 concentrations and assessing their levels according to the cut-off values. The possible benefits also include the evaluation of CH subtype. Nevertheless, the reference range of thyroid hormones varies all around the world and impedes the introduction of universal testing recommendations. Unification of the methodology in neonatal screening would be advantageous for prevention and management of CH. Current guidelines recommend levothyroxine treatment of CH in preterm infants only when the diagnose is confirmed. Moreover, they underline the importance of the re-evaluation among preterm born infants due to the frequency of transient forms of hypothyroidism. However, results from multiple clinical trials are mixed and depend on the newborn's gestational age at birth. Some benefits of treatment are seen especially in the preterm infants born <29 weeks' gestation. The discrepancies among trials and guidelines create an urgent need to conduct more

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large sample size studies that could provide further analyses and consensus. This review summarizes the current state of knowledge on congenital hypothyroidism in preterm infants. We discuss screening and treatment options and demonstrate present challenges and controversies.

Keywords: congenital hypothyroidism, hypothyroxinemia, neonatal screening, preterm newborns, thyroid hormones

INTRODUCTION

Thyroid hormones (TH) play a significant role in the development of every neonatal organ, especially brain. Insufficient maternal TH levels during the first trimester of pregnancy are associated with numerous disfunctions noticeable before, right after birth and later in an adult life (1). However, it is commonly known, that fetus is dependent on the maternal TH supply only until the end of the first trimester (2, 3). In the eighth week of gestation fetal hypothalamus, as well as fetal gut and pancreas in a lesser degree, begin to produce the thyrotropin-release hormone (TRH), which stimulates a pituitary gland to secrete the thyroid-stimulating hormone (TSH). In the tenth week fetal thyroid starts to accumulate iodine, produce thyroglobulin, and express TSH receptors. Simultaneously, fetal thyroxine-binding globulin (TGB), TSH and T4 levels begin to increase and double up until the term. Serum total T4 concentrations reach a value of 130 nmol/L with plateau at 35-37 weeks (4). The hypothalamic-pituitary-thyroid axis starts to mature by the second trimester of gestation. According to the new consensus by the European Society for Pediatric Endocrinology and European Society for Endocrinology, congenital hypothyroidism is defined as the hypothalamic-pituitary-thyroid (HPT) axis's insufficient development, which is particularly observed in premature newborns, and results in numerous complications including an impaired thyroid activity and inadequate TH secretion (**Figure 1**) (5).

Statistic data suggests that globally 10,6% of births occur before 37 weeks' gestation, which leads to about 15 million preterm births every year. Four percent of them occur before completed 28 weeks of gestation, while moderate to late preterm births (at 32-36 completed weeks) stand for 84% (6). Although many factors have been proven to increase the risk of spontaneous preterm birth, the vast majority of them appear in women without a clear risk factor. However, there is strong evidence that elevated level of maternal TSH may result in pregnancy loss or preterm delivery (7–9). It indicates that

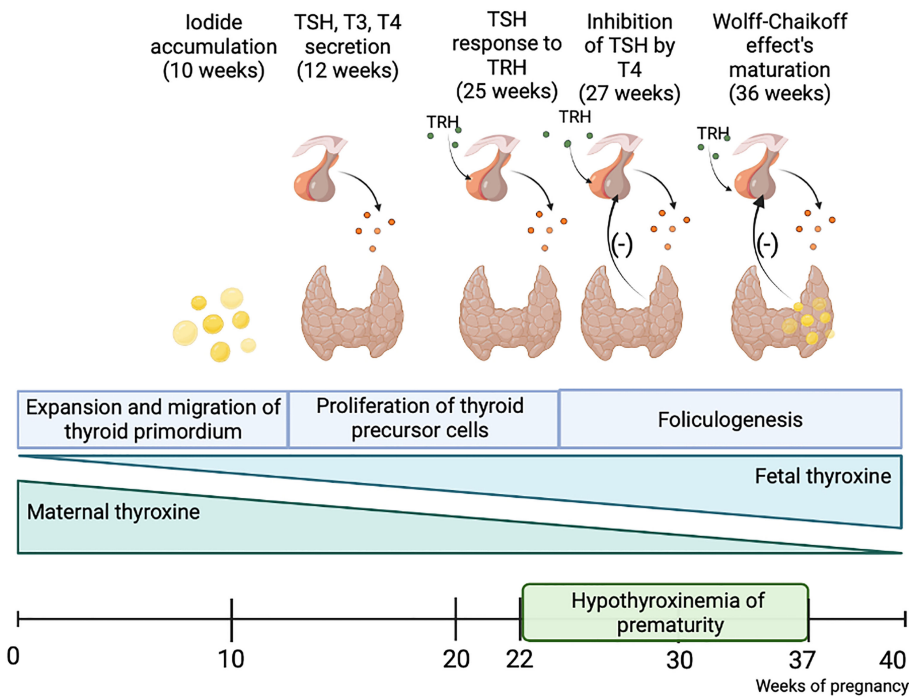

FIGURE 1 | Fetal thyroid gland maturation. The fetus is dependent on maternal thyroid hormones supplementation until the end of the first trimester. The hypothalamus-pituitary-thyroid axis is not sufficiently developed until the end of pregnancy, thus preterm-born children present a disorder called hypothyroxinemia of prematurity, which results in numerous consequences. Created with BioRender.com.

thyroid hormones play a significant role in not only the development of fetal organism, but also have a huge impact on the pregnancy outcomes.

The prevalence of congenital hypothyroidism (CH) in general population is estimated between 1:2000 and 1:3000 newborns (10). Multiple screening programs confirm its higher incidence in preterm infants, with almost 50% occurrence (11–14). According to the data mentioned above, along with the week of gestation, the development of the hypothalamic-pituitary-thyroid axis accelerates. Because of that, clinical manifestations and the severity of thyroid disorders variate depending on the premature newborn's gestational age (15). The main differences involve diverse thyroid hormonal levels, whose insufficiency induces neurodevelopmental deficits (16). The detrimental implications of these comorbidities contribute to the urgency of an effective treatment. With various possible therapeutic choices and their unanticipated outcomes, there is a great need for further research and analysis (17).

In this study our aim is to explore and analyze the foundations and indications of thyroid disorders in premature newborns, the treatment options, and possible challenges. The review consists of presentation and analysis of literature and previously published studies obtained from PubMed, Scopus, and Google Scholar databases.

CAUSES OF THYROID DISORDERS IN PRETERM NEONATES

The complexity of neonatal thyroid disorders is generally known and still to be thoroughly classified. Evaluated risk factors involve for instance advanced maternal age, medication during pregnancy, family history of thyroid disease, low birth weight, preterm birth, twin pregnancy or birth defects (18, 19). Due to the serious general condition of premature infants, there is a necessity for both a careful perinatal care and further clinical analysis (20).

Maternal Causes of Thyroid Disorders

One of the most frequent bases of the newborn's thyroid abnormalities is in the mother's endocrine disorder. The association between isolated maternal hypothyroxinemia and preterm birth is currently observed (21). In 2019 Korevaar et al. published a meta-analysis concerning the hypo- or hyperthyroidism of mother correlating with infant's thyroid function and preterm birth. After including 19 cohorts with a population of 47 045 pregnant women, it was stated that patients' subclinical hypothyroidism, isolated hypothyroxinemia and TPO antibody positivity directly affect higher risk of preterm birth (22). Maternal subclinical hypothyroidism is also associated with the lower birth weight as well as newborns small for gestational age (SGA), which explains the need for a careful therapy during pregnancy (23, 24). However, in 2018 Varner et al. conducted multi-center randomized, double-masked, placebo-controlled thyroxine replacement trials in pregnancies in order to assess the probable neonatal benefits of maternal subclinical hypothyroidism/hypothyroxinemia treatment. The conclusions stated no statistically relevant difference in infants' TSH levels, which questions the value of prebirth pharmacotherapy (25). Contrary, current guidelines emphasize the importance of treating maternal overt hypo- and hyperthyroidism both during pregnancy and before the conception. What is more, the recommendations favor implementing targeted screening in case of high-risk pregnancies rather than universal screening for thyroid disorders before or throughout the pregnancy (26).

Maternal thyroid disorders not only affect the duration of pregnancy and labor, but also directly influence the newborn's neuropsychological development. Studies show that low maternal free thyroxine concentrations may impair infant's psychomotor and cognitive abilities leading to underperforming in school, learning difficulties or even behavioral and emotional disorders (27). Authors specifically examine Graves's disease in mothers' association with thyroid disfunction and SGA in neonates (3, 28). As the disorder not only remains the main cause of hyperthyroidism in pregnant women, but also bears risk of severe consequences, it needs to be diagnosed and adequately treated as soon as possible (29).

Pregnancy Complications

Preterm labors may occur as a result of placental abruption or insufficiency or other pregnancy-related complications, especially in hypo/hyperthyroid women (30, 31). Endangered pregnancies have been identified as a significant factor in preterm neonatal thyroid disorder. Pre-eclampsia carries a great risk of placental insufficiency, which may induce intrauterine hypothyroidism (32). It is also claimed that perinatal asphyxia results in lower TSH, T4, T3 and FT4 cord blood levels in newborns (33).

Genetic Factors and Thyroid Malformations

Generally, thyroid disorders in preterm newborns occur spontaneously or because of previously mentioned risk factors. Nevertheless, plenty of studies yield information about familial hypothyroidism with prevalence up to 2% (34). The genetic etiology of the disease seems to explain extrathyroidal malformations reported in some cases of CH with numerous candidate genes to possibly be responsible (34–36).

Mentioned thyroid morphological defects are known as a separate risk factor for congenital hypothyroidism in preterm newborns (37). Total or hemi agenesis, ectopy and hypoplasia are objectively diagnosed in up to 85% cases of thyroid dysgenesis (36).

Pre- and Postnatal Medications

Multiple medications implemented during pregnancy and in the postnatal stage have numerous diverse effects on infants. However, in most cases their usage is a necessity, thus possible consequences must be considered. Amiodarone, which is commonly used in pregnant women for treatment of maternal and fetal dysrhythmias, may cause infant's hypothyroidism (38). The drug is rich in iodine and resembles thyroxine in structure, so its administration may alter thyroid function (39). In preterm newborns the impact of excess iodine from amiodarone on TH is linked to the disturbed Wolff-Chaikoff effect (40). In general, the oversupply of iodine inhibits not

only its organification but also thyroglobulin proteolysis (41). Recent studies show that 18,2% of premature neonates administered with iodinated contrast media (ICM) had transient hypothyroidism (42, 43). Nevertheless, a trial by Rath et al. suggests that ICM may cause adverse thyroid effects only when its administration is not conducted carefully (44). Moreover, lower TH levels are observed in preterm breastfed infants after their lactating mothers had a CT scan with ICM (45). Bowden et al. described the inhibition of TSH release induced by glucocorticoids, somatostatin, and dopamine (46). However, Ekmen et al. suggests that TSH, T3 and T4 levels are not disturbed during dopamine infusions (47). Bearing in mind that dopamine is known to suppress thyrotropin release, there is an urgent need to conduct more trials concerning its impact on infant's TH. Some studies emphasize the negative influence of glucocorticoids on TSH and thyroxine secretion. The trial by Shimokaze et al. suggests that very short-term glucocorticoid administration may cause marked changes in TH levels (48).

THE EFFECTS OF THYROID DISORDERS IN PRETERM INFANTS

The thyroid gland produces triiodothyronine (T3) and thyroxine (T4) in response to pituitary gland stimulation (46). In cells T4 converts to T3 so that biofeedback mechanism maintains adequate levels of thyroxine for body metabolism and, in children, a proper growth and brain development. Thyroxine is a vital necessity for all the organs, tissues, and cells in the body to function normally. It also controls the body's metabolic rate and other multiple processes (49) (**Figure 2**).

Thyroxine deficiency in early neonatal period may cause severe, irreversible mental and physical retardation, a condition known as cretinism. It is worth mentioning that fetal and newborn periods are the exact brain development stages when differentiation of numerous structures occurs in a short amount of time. Even small or subtle alterations in thyroid hormones levels may result in a disturbed brain development and a long-lasting or permanent deficits. As said before, fetus is dependent on maternal TH supply until the end of the first trimester (2, 3). Thus, during that period all the TH concentrations in fetal tissues are of maternal origin and even a small disruption in their transfer may result in brain development disturbances. Nevertheless, the fetus's endocrine system begins to mature in early stages of gestation (50). On the other hand, CH is a type of TH deficiency which starts in late gestation and is caused by low TH fetus/infant production. Recently, multiple studies showed that TH have a profound role in oligodendrocytes and astrocytes' maturation. For instance, T3 regulates cerebral cortex stratification, axon routing and cell migration by Cajal-Retzius and subplate cells (51). What is more,

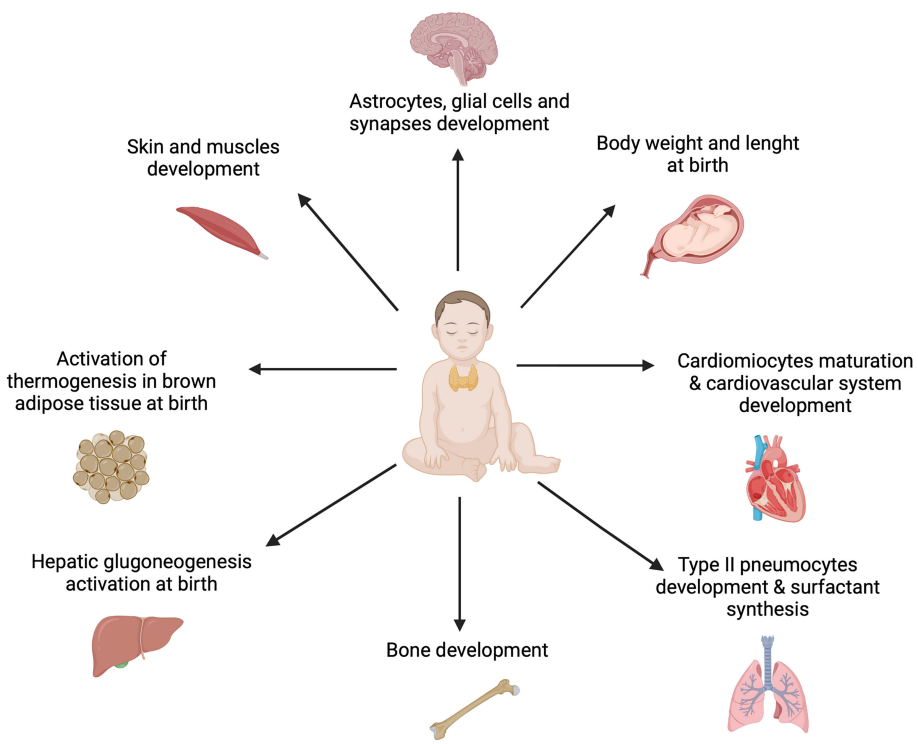

FIGURE 2 | The role of thyroid hormones in fetal and infant development. Thyroid hormones are essential for the general accretion of fetal mass and to provoke developmental events in the fetal brain and somatic tissues from early in gestation. Moreover, they affect the production of other hormones and regulate tissue accretion near term. Furthermore, TH ensure activation of physiological processes as thermogenesis, gluconeogenesis, pulmonary gas exchange and cardiac adaptations at birth (49). Created with BioRender.com.

TH upregulate the differentiation and expression of neural cells as well as play a role in myelination and synaptogenesis (52, 53). De Souza Martins et al. demonstrated the positive influence of T3 supplementation on Myosin-Va (Myo-Va) expression. Myo-Va is a molecular motor protein that affects vesicle and RNA carriage. Its malfunction leads to abnormal axonal transport and synaptic function (54). Moreover, low TH levels affect glial cells. Opazo et al. presented that gestational hypothyroxinemia affects neural reactivity, causing its decrease in microglia and increase in astrocytes, while Flamant et al. suggested the link between a T3-dependent neurotropin secretion and oligodendrocyte progenitor differentiation (55–57). Furthermore, the synthesis of extracellular matrix proteins, which is under the TH control, has been linked to a reduced cortical thickness (57). Currently, the research concerning the role of TH in brain development gathers pace. However, the evidence is still limited, thus there is an urgent need to conduct more clinical trials.

Epidemiology

Considering the remarkable progress and innovations in modern medicine, the survival rate of preterm newborns is constantly escalating (58). Although proper neonatal care should enhance neonates' healthy development, in many cases it is insufficient (59). Consequently, there is an increasing incidence of congenital hypothyroidism (60, 61). Despite its estimated prevalence between 1:2000 and 1:4000 in infants, there is evidence about epidemiological differences dependent on the geographic location (62).

Classification of Congenital Hypothyroidism in Preterm Newborns

Congenital hypothyroidism is a condition occurring in infants unable to produce a sufficient amount of thyroid hormone (thyroxine, T4), which is necessary for a normal metabolism, growth and brain development. The disorder develops sporadically and is rarely inherited.

Primary congenital hypothyroidism (PCH) is the most prevalent form of CH, because of which the serum T4 levels are low, whereas TSH levels are elevated (63). Moreover, it is not associated with neither prenatal nor risk factors. There are several types of primary CH, the most common form being caused by abnormal fetal development of the thyroid gland (36). Severe PCH entails the highest risk of neurodevelopmental impairment (64).

Permanent congenital hypothyroidism develops as a result of perpetual dyshormonogenesis or thyroid dysgenesis (65). Its risk factors are not fully analyzed yet (66). The prevalence in neonatal screenings is estimated at 1:748 (67). Surprisingly, it appears similarly in both pre- and term infants (68). As oppose to transient CH, it is said to occur more often in women than in men (69).

Transient congenital hypothyroidism (TCH) is more common in preterm neonates, with incidence of 1:1114 (67). The syndrome may develop as a result of maternal exposure to antithyroid medications or fetal exposure to maternal antithyroid antibodies (65, 70). What is more, the use of iodine-based skin disinfectants on premature infants can inhibit thyroxine production resulting in transient hypothyroidism (71). Untreated maternal hypothyroidism might lead to low fetal levels of thyroxine as well (71). TCH is characterized by decreased levels of thyroid hormones evaluated at birth which return to normal values after a few months or years of life (72).

Secondary or central congenital hypothyroidism (CHC) is observed in children after brain damage, intraventricular hemorrhage and in cases of an insufficiency of hypothalamus or/and pituitary gland (73). This disorder can only be diagnosed using screening tests detecting TSH and fT4 levels simultaneously or stepwise (74). As a matter of fact, CHC must be distinguished from T4-binding globulin deficiency (75).

Hypothyroxinemia of Prematurity

In premature neonates, serum TSH, T4 and free T4 (fT4) concentrations tend to change dynamically according to their postmenstrual age (PMA). The initial high TSH values (with the peak at PMA=30 weeks) gradually decrease to reach the term infants' level at PMA=38-40 weeks. Adequately, the T4 and fT4 levels are the lowest around PMA=26-27 weeks, only to also reach the norm at PMA=38-40 weeks (76, 77). Moreover, lower TGB concentrations are responsible for the decreased T4 serum levels.

Furthermore, some very low birth weight and extremely low birth weight premature neonates present delayed TSH elevation (dTSH), which is also known as "atypical hypothyroidism" (78–80). It occurs when second capillary screening at 1 month of age shows serum TSH concentrations higher than 20 mIU/L following a normal value below 15 mIU/L at first neonatal screening within first 4-6 days after birth (81). Woo et al. conducted a study to establish the prevalence of dTSH. The results identified the incidence of 1:58 in ELBW and 1:95 in VLBW infants. In comparison, it estimated 1:3029 in newborns with weight higher than 1500 grams, which proves dTSH dependence on low birth weight (82).

Transient hypothyroxinemia of prematurity is a term regarding the time of premature infant's life with serum low thyroid hormone levels. The decreased values are suggested to originate from the immaturity of the hypothalamic-pituitary-thyroid axis (83). Studies mention various risk factors for this abnormality: lower gestational age, maternal pre-eclampsia, respiratory distress syndrome, mechanical ventilation, and dopamine infusions (32, 84, 85).

Although hypothyroxinemia of prematurity is a separate disorder, and not an additional form of congenital hypothyroidism, authors usually do not differentiate between them. Both conditions prevail because of preterm infants' insufficient fetal thyroid evolution and multiple other risk factors described before (86). Clinical manifestations of the two diseases are very similar due to their strict origin from prematurity, thus it is almost impossible to classify them accordingly. Current guidelines do not specifically recommend treating hypothyroxinemia of prematurity with levothyroxine, unless TSH elevation is also observed (87). However, because of considerable difficulty in the exact diagnosis, it is usually treated as CH up to the necessary reevaluation after 6 months of life (5).

Iodine deficiency can be observed in preterm infants due to its insufficient amount in the parenteral nutrition and the rapid loss

of maternal supply. It contributes to slow recovery from hypothyroxinemia of prematurity. Therefore, the newborn's exposure to excess iodine, for instance iodine disinfectants or radiological contract infusions, leads to down-regulation of T4 and T3 levels, also known as Wolff-Chaikoff effect (71).

NEONATAL SCREENING IN GROUP OF PRETERM NEONATES

The early diagnosis of congenital hypothyroidism in preterm newborns is a pivotal factor conditioning the patient's neuropsychological and motor development (88). Neonatal screening tests are most commonly performed by sampling blood from infant's heel between the 2nd and 5th day after birth and evaluating the TSH, T4 and fT4 levels by using fluoroimmunoassay (89). The implemented TSH cut-off levels vary all over the world which provides different epidemiological reports and impedes the introduction of universal neonatal screening guidelines (90). For instance, in Thailand the optimal cord blood TSH value for recall is estimated at 30-40 mIU/L, while in Italy it stands at 10 mlU/L (91, 92). Kilberg et al. argue that TSH cut-off values in neonatal screening should be age-adjusted in order to detect mild cases of CH and persistent TSH elevations (93). The repetition of screening is said to be beneficial for the assessment of infant's condition and treatment effects as well as classification of CH, permanent or transient, that allows an adequate patient's management (94). Follow-up examinations are advised to be done after four, eight weeks and later every three months (90). Post screening strategies involving collecting of second blood specimen between 10th and 14th days after birth are recommended in neonates at risk of CH, which is preterm, low birthweight and sick infants. The mentioned newborns may present false-negative results in first neonatal screening (5).

The congenital hypothyroidism occurs when the detected TSH level is higher than the cut-off value and fT4 level is decreased. The pharmacotherapy with levothyroxine needs to be implemented. With tablet form the dose should be at 10-15 μg/kg/day and the medication should be taken 60 minutes before the breakfast (95, 96). In case of choosing the liquid form, the dosage should be the same, if not lower, as the suppression of TSH is higher due to higher absorption (97–99). Additionally, the liquid levothyroxine dissolves without an acid gastric pH, so it can be administered along with the meal (96). If TSH concentration is estimated within the range of the reference and cut-off values along with the decreased fT4 level, the diagnosis and treatment plan stand exactly as mentioned above. With normal TSH and fT4 values we can identify a proper thyroid function which does not require any medications. In case of decreasing of both TSH and fT4 concentrations, secondary CH is suspected and should be treated with levothyroxine at a dose 5-10 μg/kg/day or lower, at 1-2 μg/kg/day, in children with gland *in situ* or with isolated secondary CH (5, 90). In recently published Consensus Guidelines by an ENDO-European Reference Network Initiative Endorsed by the ESPE and ESE, if the serum TSH level estimates between 6-20 mU/l and fT4 concentration is within age specific references, or if the TSH value is higher than 20 mU/L and fT4 concentration is below the age-specific references, levothyroxine replacement therapy is immediately needed (5). Presented recommendations for dosages and values may differ regarding other guidelines, thus there is an emphasized need for unification of universal screening approaches.

THE THYROID HORMONE REPLACEMENT THERAPY IN PRETERM NEWBORNS

Described above numerous complications of congenital hypothyroidism indicate a tremendous need to conduct a therapy able to alleviate the course of the disease. Current guidelines recommend the supplementation of levothyroxine preparations (L-T4) in children with congenital hypothyroidism (5, 90). However, recent studies have yielded plenty of information on different outcomes of the thyroid hormone replacement therapy, both in preterm born children with transient hypothyroxinemia and CH (100–106). Indeed, here we describe current guidelines and the newest results of the clinical trials.

Current Guidelines and Recommendations

Levothyroxine is known to be the most effective drug in congenital hypothyroidism. The crucial part of the treatment is the exact time of its initiation. Guidelines recommend starting the therapy not later than on the 14th day of life. SGA, VLBW and ELBW newborns have greater risk of CH and its consequences, thus the time of the effective drugs action is short-up to four weeks after labor. Guidelines suggest maintaining the fT4 and fT3 levels not later than in 1-2 weeks. The initial dose of L-T4 depends on the severity of the disease. In primary CH levothyroxine in tablet form starts from 10–15 μg/kg/day and increases in children with severe CH (5). Oral administration on an empty stomach at least 30 minutes before eating is recommended (95, 96). Levothyroxine in liquid form can be administered along with the meal and the dosage is evaluated the same or lower than in the tablet form (96–99). The target dose should be adjusted according to the serum levels of TSH and fT4 to ensure stable euthyreosis. In suspected central CH levothyroxine dose is estimated at 5-10 μg/kg/day and in diagnosed CHC at 1-2 μg/kg/day. Reevaluations beyond the first 6 months of life are crucial to assess the need or its absence for further therapy (5). TSH level should be within the range of the reference values for age, and fT4 in the upper half of them. It is worth mentioning that inappropriate dose of levothyroxine (both insufficient and excessive) may cause multiple adverse effects and disturb treatment's effects (107). The check-ups should be performed within specific time spans and accordingly to the patient's needs (5, 90).

Clinical Trials Do Not Correspond With Each Other

Some studies correspond with guidelines and show that preterm infants supplemented with levothyroxine perform significantly better in both cognitive and motor functions (100–102). However, as the research on that topic had been gathering pace, more studies not correlating with neither the previous ones nor the

recommendations had emerged (104–106) (**Table 1**). In 2020 Ng et al. enrolled 153 infants before 28 weeks' gestation to an explanatory double-blind, randomized, placebo-controlled trial. Children were supplemented with L-T4 or given the placebo until 32 weeks' corrected gestational age. Neurodevelopmental outcomes after 42 months showed that the L-T4 supplemented group performed significantly better in motor, language, and cognitive function domains (100). Accordingly, in 2014 Noumra et al. showed that L-T4 supplementation prevents the developmental delay of extra low birth weight infants with transient hypothyroxinemia. Moreover, the trial proved the association of gestational age with serum levels of fT4 (101). The study by Suzumura et al. demonstrated that levothyroxine treatment in extremely preterm newborns initiated at the end of the first week of life could reduce the incidence of cerebral palsy (102). However, Van Wassenaer-Leemhuis et al. did not find any differences in mental or motor development and rates of cerebral palsy between the compared groups of infants born less than 28 gestational weeks, treated or not with levothyroxine (106). Van Wassenaer et al. reported there is no correlation between the initial plasma free thyroxine concentration and the effect of treatment. In the study 200 infants born before 30 weeks' gestational age received orally L-T4 or placebo treatment for 6 weeks. The follow-up did not show any differences in mortality or morbidity between compared groups. In thyroxine-treated infants born before 27 weeks' gestation the Mental Development Index measured at the age of 24 months was 18 points higher than in the placebo group, while among children born 27 weeks or later the same index was 10 points lower in the treated group than that of their counterparts (103). Hollanders et al. suggest no association between transient hypothyroxinemia of prematurity and neurodevelopmental outcome in young adulthood. The study was a part of 19 years follow-up project which included infants born very preterm and with very low birth weight. This long-time multicenter trial demonstrated no correlation of IQ score or motor function with hypothyroxinemia in preterm born children after adjustment for demographic and perinatal characteristics (105). Yoon et al. aimed to determine the incidence, etiology, and outcomes of the TSH elevation and its treatment in extremely low-birth-weight infants (ELBWIs). Indeed, the levothyroxine replacement therapy resulted in significantly higher TSH elevations, lower fT4 levels and

TABLE 1 | Summary of studies concerning the T4 treatment and its neurodevelopmental outcome.

Study	Intervention	GA	Total Group T4 vs placebo	Endpoint	Main results
Clinical trials which correspond with current guidelines					
Ng et al. (100)	T4, daily bolus, first 5 days iv, later orally; 8 µg/kg until 32 weeks' corrected GA	<28 weeks	61 vs 57	Neurodevelopment at 42 months	Supplemented group performed significantly better in motor, language, and cognitive function
Nomura et al. (101)	T4, daily bolus; 5-10 µg/kg orally	— ELBW infants	18 vs 18	Neurodevelopment at 12 months corrected age	T4 prevents the developmental delay of ELBW infants
Suzumura et al. (102)	T4, daily bolus; 5-10 µg/kg for FT4 levels <0.8 ng/dL	<28 weeks	54 vs 60	Cerebral palsy at 3 years	Reduction of cerebral palsy incidence
Ben-Skowronek & Wisniowiecka (110)	T4, 5-10 µg/kg b.w./day since the second week of life	25-35 weeks LBW, VLBW ELBW	40 vs 52	Mental development in the 7th year of life	Improvement in long-term mental development
Clinical trials which do not correspond with current guidelines					
Vanhole, et al. (111)	T4, daily iv bolus, 20 µg/kg, d 1-14	<31 weeks	17 vs 17	Endocrine and clinical manifestations during first 2 weeks of life; Neurodevelopment at 7 months	No difference in clinical outcome and development
Van Wassenaer et al. (112)	T4, daily bolus, first 2-3 weeks iv, later orally; 8 µg/kg, d 1-42	<30 weeks	82 vs 75	Neurodevelopment at 24 months	No difference in total groups. Subgroup analyses: at 2 and 5yrs better outcome with T4, if Ga <27-29 weeks
Smith et al. (113)	T4, bolus, start iv: 10 mg/kg; then orally: 20 µg/kg, d 2-21	<32 weeks	29 vs 18	Chronic lung disease; Oxygen dependency at day 28	No effect on the incidence of chronic lung disease
Briet et al. (114)	T4, daily bolus, first 2-3 weeks iv, later orally; 8 µg/kg; d 1-42	<30 weeks	82 vs 75	Neurodevelopment at 24 months; Motor and neurologic outcome at 5.7 years	No difference in total groups. Subgroup analyses: at 2 and 5 years better outcome with T4, if Ga <27-29 weeks
Biswas et al. (115)	T3, continuous iv, 6 µg/kg/d + hydrocortisone 1 mg/kg/d; d 1-7	<30 weeks	125 vs 128	Death or ventilator dependence at day 7	No difference in adverse outcome
Van Wassenaer-Leemhuis et al. (106)	T4, daily bolus; 4-8 µg/kg iv or iodine 30 mg/kg iv; d 1-42	<28 weeks	14 (iodine) vs 62 (T4) vs 13	Cerebral palsy, mental and motor development at 3 years	No difference among groups
Yoon et al. (104)	T4, daily bolus; 10-15 µg/kg until the TSH normalized levels	>23 weeks and with ELBW	25 vs 22	Growth and neurodevelopment at 2 years	No difference among groups

GA, gestational age; ELBW, extremely low birth weight; T4, thyroxine; iv, intravenous; d, day.

significantly reduced mortality compared to untreated children. Nevertheless, according to the follow-up, the treatment had no significant effect on neurodevelopmental outcomes and growth (104).

Guidelines do not recommend the use of iodine in congenital hypothyroidism therapy (5, 90). However, some trials, regarding to iodine's essential role in the synthesis of thyroid hormones, try to investigate whether its dietary supplementation affects thyroid function during the neonatal period. The meta-analysis performed by Walsh et al. did not show any effect of iodine intake on mortality or neurodevelopment in two-years follow-up. However, analyzed trials assessed the effect of prophylactic rather than therapeutic iodine supplementation (108). Further research conducted by Ares et al. revealed similar results. Ninety-four infants with very-low birth weight were enrolled to the trial and assigned into two groups. Children in the intervention group were treated everyday with iodine in oral drops, while the placebo group did not receive any supplements. Blood samples were collected for thyroid hormones and the neurodevelopment was assessed. The analysis showed a positive outcome on the blood levels of thyroid hormones. Infants in the supplemented group reached the recommended levels from the start of the trial. Nevertheless, positive neurodevelopmental effects of iodine intake were not found. The study suggest that preterm newborns are at high risk of iodine deficiency, thus their iodine intake should be monitored. Iodine supplementation should be considered if the intake is found to be insufficient (109).

CONCLUSIONS

Despite considerable progress of management and treatment of congenital hypothyroidism, the disorder remains to induce substantial failures in infants' neurodevelopment. An efficient solution that could influence not only a course of CH, but also its implications, is a proper establishment of how TH affects an infant's brain, especially during pregnancy and early childhood. CH originates from multiple factors, thus their elimination could contribute to decreasing the prevalence of the disease. Unfortunately, there is still some uncertainty considering the effects of pre- and postnatal treatment of prematurity that could induce CH. In these cases, the avoidance of said risk factors seem almost impossible and require further evaluation and research. Moreover, the prognosis and possible therapeutic outcomes are crucially dependent on the early diagnosis of CH.

It is vital to identify the subtype of CH in preterm infants, as the exact classification enables an effective and appropriate management and treatment. Bearing in mind the frequency of transient CH, probable risk factors should be considered whereas necessary reevaluations and follow-ups ought to be implied in cases of uncertain diagnosis. We also need to be able to identify phenomena such as hypothyroxinemia of prematurity or delayed elevation of TSH, as their hormonal manifestations or implications substantially differ from typical CH forms.

Neonatal screening tests play a vital role in an effective disease recognition. Although a significant progress has been made in recent years, there is still a strong need for reevaluating and unifying screening guidelines to achieve coherent CH management and therapy. Authors specifically draw attention to mild cases of CH in which are sometimes impossible to detect. As mentioned before, reevaluations are crucial in cases of transient CH in order to assess the patient's status and potential need or its absence for further therapy.

Current treatment guidelines recommend thyroid hormones substitution in children with congenital hypothyroidism. However, clinical trials have yielded plenty of information about diverse therapeutic results. Authors still aim to assess the most appropriate clinical approaches and dosages of levothyroxine. What is more, treatment models differ between studies and guidelines, thus comparing and analyzing their effects remains problematic. There are also questions considering the iodine supplementation. Although the guidelines do not recommend the use of iodine in the therapy of CH in preterm infants, it appears to be a subject of clinical trials and a possible addition to prevention. It is essential to conduct more research considering the therapy of CH in premature newborns as to unify the expected outcomes.

So far, medical society has gained plenty of up-to-date and thorough knowledge about congenital hypothyroidism in preterm infants. However, analysis of literature and current challenges presented in this review prove the urgent demand for further research.

AUTHOR CONTRIBUTIONS

MK contributed to the conception and design of the work, acquisited and analyzed of references for the work, and wrote the first draft the manuscript. AK contributed to the conception and design of the work, acquisited and analyzed of references for the work, and wrote the first draft the manuscript. IB-S contributed to the conception and design of the work, acquisited and analyzed of references for the work, and drafting the work or revising it critically for important intellectual content. All authors contributed to manuscript revision, read, and approved the submitted version the manuscript.

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Comparison Among Two Liquid Formulations of L-thyroxine in the Treatment of Congenital Hypothyroidism in the First Month of Life: A Pilot Study

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The liquid formulation of L-thyroxine is the most used in the substitutive treatment of congenital hypothyroidism (CH). This formulation has higher TSH suppression rates with respect of L-thyroxine tablets and thus lower doses are indicated. Two types of liquid L-thyroxine (Tirosint© and Tifactor©) are currently approved in Italy for use in pediatric age and to date there are no data available in the Literature comparing the two liquid formulations. The aim of this study is to compare the efficacy of both formulations in normalizing TSH and fT4 levels in the first month of life and to compare the L-thyroxine requirement for both formulations over the same period. All newborns diagnosed with primary CH at the neonatal screening program in the Piedmont region of Italy in the period May 2020 – May 2021 were enrolled and divided into two groups according to the liquid formulation used: TS Group with Tirosint© and TF Group with Tifactor©. No difference was observed between the two groups considering the TSH at dried blood spot (DBS) at neonatal screening, the serum levels of TSH, fT4 and fT3 and initial dose of L-thyroxine. At 15 days the serum TSH in the TF Group was 0.08 ± 0.02 mcUI/ml, while in the TS Group it was 36.7 ± 14.7 mcUI/ml p=0.04). No differences were observed between the two groups considering fT4 levels and L-thyroxine requirement. Among the subjects in the TF Group, 5/9 showed suppressed TSH at 15 days after starting treatment, while none of the subjects in the TS Group showed TSH levels below the normal lower limit (p=0.011). Among the subjects in the TF Group, 5/9 patients showed suppressed TSH at 30 days after starting treatment, while 1/12 subjects in the TS Group showed TSH levels below the normal lower limit (p=0.017). In conclusion, this study confirms the efficacy in normalizing the thyroid hormonal profile in newborns with CH among the liquid solutions although the response seem to be different in timing therefore an individual approach is necessary considering the type of formulation used, the diagnostic category of CH and clinical features.

Keywords: congenital hypothyroidism, liquid L-thyroxine, newborns, pediatric age, treatment

INTRODUCTION

Congenital hypothyroidism (CH) is the most common endocrine disorder in pediatric age (1). Prompt diagnosis and treatment are fundamental to avoid neuro-developmental delay, thus neonatal screening strategies are implemented in most countries. In recent years, the incidence of CH has increased worldwide due to many factors such as the lowering of the TSH cut-off detection at neonatal screening, the increase of the neonatal population at risk for developing CH and genetic-environmental factors. This led to the change in incidence in the Piedmont region in Italy from 1:3000-4000 in the '90s and 1:2200 in the first decade of 2000 to the current 1:1090 (2). Once reported by the national screening center, the diagnosis of CH should be confirmed by evaluating the thyroid hormone profile of thyroid stimulating hormone (TSH), free Thyroxine (fT4) and free triiodothyronine (fT3), thyroglobulin dosage and radiological evaluation by ultrasound and/or Tc^{99} or I^{121} scintiscan. The titer of Anti-peroxidase (AbTPO), anti-thyroglobulin (AbHTG) and anti-TSH receptor antibodies should also always be determined at the time of diagnosis.

The latest guidelines indicate an initial dose of L-thyroxine at 10-15 mcg/kg/day, to be started within the first two weeks of life in case of severe CH and within the first month in milder conditions (1). Synthetic L-thyroxine has a comparable structure to T4, and the substitutive treatment can be started in tablet or liquid formulation (3). The solid formulation requires an acid gastric pH to be dissolved, thus its administration should take place 20-30 minutes before breakfast (4). The liquid preparation is bioequivalent to the solid formulation and does not need acid gastric pH for the dissolution phase due to its liquid physical state (5, 6).

Currently, the most used formulation in Italy in newborns is a liquid formulation administered in drops containing 3.57 mcg of L-thyroxine and 8.68 mg of ethanol (Tirosint©, Ibsa Farmaceutici, Lodi, Italia, approved in Italy since 2009). Since 2019 another L-thyroxine liquid formulation has been approved in Italy for use in the pediatric age (Tifactor©, Galenica Pharmaceutical Industry, Athens, Greece), containing 20 mcg of L-thyroxine in each ml and glycerol, but not ethanol. Chronic administration of ethanol does not represent a contraindication to prescribing Tirosint© in the pediatric age but there is controversial concern about the potential risk of side effects from its assumption for a long period (7). Recent studies have observed a higher rate of TSH suppression in patients treated with the liquid formulation than in subjects treated with the solid formulation, exposing those patients to a greater risk of overtreatment, with iatrogenic hyperthyroidism, which may be harmful in the first years of life and increase the risk of developing attention disorders later in childhood (7–9). Therefore, some Authors indicate to use lower doses of the liquid formulation (Tirosint©) than the solid formulation (7).

To date, no data are available in the Literature comparing the two liquid formulations (Tirosint© vs Tifactor©) approved for the treatment of hypothyroidism in pediatric age in Italy. The aim of this study is to compare the effectiveness of both formulations in normalizing the TSH and fT4 levels in the first month of life and to compare the L-thyroxine requirement for both formulations over the same period.

MATERIALS AND METHODS

All newborns diagnosed with primary CH, detected at the neonatal screening program in the Piedmont region in Italy in the period May 2020 – May 2021, were enrolled. All the TSH detection tests on dried blood spot (DBS) were performed at the regional reference center for Neonatal Screening at Regina Margherita Children's Hospital, in Torino, Italy.

Infants with syndromic CH or chromosomal abnormalities, transient congenital hypothyroidism (TCH), or isolated hyperthyrotropinemia in which replacement treatment was not initiated have been excluded. Confirmation of the diagnosis of CH was based on serum TSH, fT4, fT3 and thyroglobulin levels, and on radiological evaluation with Tc99 scintigraphy and ultrasound examination in all cases of suspected thyroid agenesis. AbTPO and AbHTG antibodies were evaluated in case of unknown or positive maternal antibodies titer. All blood tests were performed in a single laboratory.

The patients were divided into two groups according to the liquid formulation used: TS Group using Tirosint© and TF Group using Tifactor©. The choice of the liquid formulation was based on the discretion of the clinician and the wishes of the family. After the start of the treatment, both groups were evaluated according to the recent Guidelines, at 15 days and 1 month, by TSH and fT4 assays, as well as with the analysis of auxological parameters (weight, length, and head circumference).

Statistical analyses and graphs were performed through Graphpad 7 software (GraphPad Software, La Jolla, CA, USA), using T-student test to compare the means and the chi-square test to compare the differences between groups.

The study was performed according to the guidelines of the Declaration of Helsinki and received the approval of the Ethics Committee of the Hospital.

RESULTS

During the study period, 21 newborns (M=8, F=13) diagnosed with CH were enrolled. Demographic and clinical data are represented in **Table 1**.

Substitutive treatment with Tifactor was started in 9/21 patients (TF Group), while Tirosint was administered in 12/21 newborns (TS Group).

No differences were observed between the two groups for sex, gestational age, neonatal weight and length, congenital malformations, co-morbidities, and maternal thyroid disease.

In the TF Group, 7/9 newborns had diagnosis of CH with eutopic gland, while ectopic gland and thyroid hypoplasia were detected in the other 2 subjects, respectively.

In the TS Group, 8/12 newborns were affected by CH with eutopic gland, while in the other 4 subjects an ectopic gland was detected.

The thyroid hormone profile and L-thyroxine requirement are represented in **Table 2**. No difference was observed between the two groups considering the TSH levels at DBS (52.11 ± 23.8 mcUI/ml in the TF Group and 102.8 ± 26.8 mcUI7ml in the TS Group, p=.19) and TSH serum levels (95.68 ± 41.1 and 138.3 ± 37.3 mcUI/ml respectively, p=0.45). The same trend was also

TABLE 1 | Demographic and clinical data of the 21 newborns with CH enrolled in the study.

	TF Group (n = 9)	TS Group (n = 12)	P value
Sex	M = 4 F= 5	M = 4 F= 8	0.60
Gestational age	39.4 ± 0.35	37.8 ± 0.68	0.08
Neonatal weight	3304 ± 168	2847 ± 181	0.08
Neonatal length	49.8 ± 0.7	48.1 ± 1.2	0.28
Congenital malformations	1/9	1/12	0.83
Comorbidities	4/9	6/12	0.80
Maternal thyroid disease	3/9	2/12	0.37
Type of CH:			
Eutopic	7	8	0.55
Ectopic	1	4	
Hypoplasia	1	0	

TF, Tifactor group; TS, Tirosint group.

observed for serum fT4 (8.94 ± 1.28 and 8.46 ± 1.48 pg/ml respectively, p=0.81) and serum fT3 (3.97 ± 0.31 and 3.86 ± 0.48 pg/ml respectively, p=0.86). The initial L-thyroxine dose was 8.88 ± 1.05 mcg/kg/day in the TF Group and 9.78 ± 0.46 mcg/kg/day in the TS Group (p=0.4).

At 15 days, the serum TSH in the TF Group was 0.08 ± 0.02 mcUI/ml, while in the TS Group it was 36.7 ± 14.7 mcUI/ml p=0.04). No differences were observed between the two groups, considering fT4 levels (21.6 ± 1.76 and 19.6 ± 1.68 pg/ml respectively, p=0.42) and L-thyroxine requirement (7.75 ± 0.92 and 8.63 ± 0.61 mcg/kg/day respectively, p=0.42).

Among the subjects in the TF Group, 5/9 showed suppressed TSH at 15 days from the start of treatment, while none of the subjects in the TS Group showed TSH levels below the lower normal limit (p=0.011). High than normal fT4 serum levels were observed in 5/9 subjects in the TF group and 6/12 patients in the TS Group (p=0.8).

At 30 days, no differences were observed between the two groups considering serum TSH (0.48 ± 0.33 and 4.98 ± 3.34 mcUI/ml respectively, p=0.26), fT4 levels (20.1 ± 1.24 and 18.6 ± 1.29 pg/ml respectively, p=0.44) and L-thyroxine requirement (5.98 ± 0.73 and 6.69 ± 0.63 mcg/kg/day respectively, p=0.47).

Among the subjects in the TF Group, 5/9 patients showed suppressed TSH at 30 days after starting treatment, while 1/12 subjects in the TS Group showed TSH levels below the lower limit of normal (p=0.017). High than normal fT4 serum levels were observed in 5/9 subjects in the TF group and 3/12 patients in the TS Group (p=0.13).

The mean TSH and fT4 levels and the mean L-thyroxine requirement at diagnosis and 15 and 30 days after the starting treatment have been represented in **Figure 1**. Subjects in the TF Group showed lower TSH levels, although statistically significant only at 15 days after starting treatment, higher fT4 levels and lower L-thyroxine dose requirement, although these parameters did not show any significant difference between the two groups.

DISCUSSION

Congenital hypothyroidism is the most common congenital endocrine disorder and the institution of prompt substitutive

TABLE 2 | Biochemical data and L-thyroxine requirement at diagnosis and after 15 and 30 days in the TF e TS groups.

	TF Group (n = 9)	TS Group (n = 12)	P value
At diagnosis:			
TSH DBS	52.11 ± 23.8	102.8 ± 26.8	0.19
TSH serum	95.68 ± 41.1	138.3 ± 37.3	0.45
fT4	8.94 ± 1.28	8.46 ± 1.48	0.81
fT3	3.97 ± 0.31	3.86 ± 0.48	0.86
L- thyroxine dose	8.88 ± 1.05	9.78 ± 0.46	0.40
At 15 days:			
TSH	0.08 ± 0.02	36.7 ± 14.7	**0.04**
FT4	21.6 ± 1.76	19.6 ± 1.68	0.42
L-thyroxine dose	7.75 ± 0.92	8.63 ± 0.61	0.42
Suppressed TSH	5/9	0/12	**0.011**
High fT4	5/9	6/12	0.80
At 30 days:			
TSH	0.48 ± 0.33	4.98 ± 3.34	0.26
FT4	20.1 ± 1.24	18.6 ± 1.29	0.44
L-thyroxine dose	5.98 ± 0.73	6.69 ± 0.63	0.47
Suppressed TSH	5/9	1/12	**0.017**
High fT4	5/9	3/12	0.15

TF, Tifactor group; TS, Tirosint group; DBS, Dry blood spot.
Significant results are in bold.

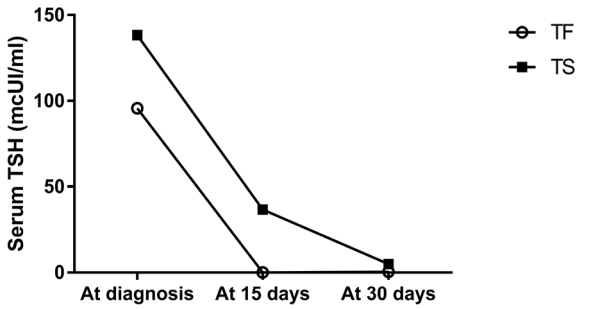

FIGURE 1 | Serum TSH change using Tirosint and Tifactor in the first month of life in newborns affected by CH.

treatment in these newborns is critical to avoid the negative effects of hypothyroidism, particularly growth and neurodevelopment impairment. The liquid formulation of L-thyroxine is currently the most used in this population, given the greater bioavailability of the oral solution compared to the solid one. Furthermore, the liquid formulation does not require acid gastric pH for the dissolution phase compared to tablets.

Previous studies have established the effectiveness of the liquid formulation, also showing a greater risk of overtreatment during the first month, possibly due to a greater absorption of this formulation than tablets (7–9). Overtreatment should be avoided, especially in the first two years of life, as it may be associated to attention deficit hyperactivity disorder (ADHD), behavioral disorders and, if prolonged, cognitive impairment in the childhood (10, 11). Therefore, some authors suggest a lower dose at the start of treatment when using liquid formulations (7).

Currently in Italy two type of liquid L-thyroxine are approved for the paediatric age. Tirosint was approved since 2009 and it is administered by drops also containing ethanol and glycerol, as excipients. Tifactor was recently approved in pediatric age as an oral solution, and does not contains ethanol.

The data from this study confirm the efficacy of both formulation in lowering TSH level and normalizing fT4 level within 15 days after starting therapy.

Currently, the initial recommended dose in CH for both Tirosint and Tifactor is the same (10-15 mcg/kg/day). We observed significant difference regarding the lowering of the TSH level after 15 days in subjects treated with the Tifactor solution compared to the newborns treated with the Tirosint drops, probably due to the slight higher bioavailability of Tifactor. No significant difference was observed in TSH level after 30 days and in fT4 levels at 15 and 30 days after initiation of treatment between the two groups, although subjects treated with Tifactor showed lower TSH level and higher fT4 level.

Tifactor dosage was lower with respect of Tirosint at diagnosis and after 15 and 30 days without significant statistical difference and with dosage titration based on TSH and fT4 levels in both cases.

In newborns treated with the Tifactor solution, we observed suppressed TSH level and higher than normal fT4 level in 55.6% of subjects at 15 and 30 days after the start of treatment. In newborns treated with Tirosint drops we observed higher than normal level of fT4 in 50% of subjects at 15 days and 25% of subjects at 30 days after the start of treatment, although these subjects showed a lower rate of TSH suppression (0/12 at 15 days and 1/12 at 30 days from the beginning of treatment).

Our data confirm the higher risk of overtreatment using liquid formulations, especially when using the Tifactor solution. This finding suggests possibly the need for a lower starting dose of L-thyroxine, when this formulation is used in newborns with CH (i.e. 7-12 mcg/kg/day).

The benefits of using Tifactor should consider the higher dosage targeting of L-thyroxine, as the minimal drug dosage change is 2 mcg, instead of 3.57 mcg when using Tirosint drops. Thus, it could be useful especially in preterm or low birth weight babies. Another factor to be considered is that Tifactor is an ethanol-free formulation, although Vigone et al. showed no side effects about growth or neurodevelopment using Tirosint drops in three-years follow-up study.

The limitations of this study are represented by the small size of the cohort and the short follow-up period. Larger cohorts and longer follow-up period are needed in further studies to determine better individualization of L-thyroxine dosage in newborns with CH and avoid the side effects of over- and undertreatment.

In conclusion, this study confirms the efficacy in normalizing the thyroid hormonal profile in newborns with CH among the liquid solutions although the response seem to be different in timing, therefore an individual approach is necessary considering the type of formulation used, the diagnostic category of CH and clinical features. Further studies in larger cohorts are needed to better determine the dose required for each of these formulations and to understand the possible influence of other factors on the different response to the therapy with the different liquid formulations.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Health and Science City University Hospital of Turin. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

GT and JM contributed to the study design, first draft of manuscript and statistical analysis. LS contributed to the study design, reference check and the revision of the final version of the manuscript. All authors contributed to the article and approved the submitted version.

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Follow-Up of Thyroid Function in Children With Neonatal Hyperthyroidism

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Introduction: Neonatal hyperthyroidism mainly occurring in the children born to mothers with Graves' disease (GD). The influence of maternal GD on the newborn's thyroid function includes not only hyperthyroidism, but also various forms of hypothyroidism. Maternally transferred thyrotropin receptor antibodies (TRAb), the antithyroid drug (ATD) administration during pregnancy and previous definitive treatment of GD (radioactive iodine therapy or thyroidectomy) in the mother impact the function of the fetal/neonatal thyroid. Some newborns born to mothers with GD may present central hypothyroidism (CeH) due to impaired regulation of the fetal hypothalamic-pituitary-thyroid axis. The aim of this study was to evaluate different types of thyroid dysfunction in babies with neonatal hyperthyroidism.

Materials and Methods: Medical records of 14 infants with neonatal hyperthyroidism (13 born to mothers with GD, and one born to mother with Hashimoto thyroiditis) were analyzed.

Results: Transient hyperthyroidism was the main thyroid dysfunction in our study group. Overt hyperthyroidism with highly increased TRAb levels (mean 13.0 ± 7.0 IU/L) was diagnosed in 6 (43%) neonates. Another 6 (43%) babies presented hyperthyroidism with slightly increased fT4 and/or fT3 levels and TSH levels in the lower limit of the normal range coinciding with positive TRAb levels (mean 3.8 ± 1.6 IU/L). Normal thyroid hormone levels with TSH levels below the lower limit of the range were observed in 2 (14%) neonates. Four babies in the study group (28.5%) required further levothyroxine (L-T4) supplementation due to CeH or, in one case, due to primary hypothyroidism.

Conclusion: Our study highlights the need for prolonged monitoring of thyroid function in children born to mothers with GD. Diagnosis of CeH could be delayed due to its masking by transient hyperthyroidism. Prolonged thyroid-stimulating hormone suppression after TRAb elimination should be considered as a signal announcing CeH.

Keywords: neonatal hyperthyroidism, central hypothyroidism, maternal TRAb, Graves' disease, neonates

INTRODUCTION

Neonatal hyperthyroidism is a rare disorder, mainly occurring in the children of mothers with Graves' disease (GD). The influence of maternal GD on the newborn's thyroid function includes not only hyperthyroidism, but also various forms of hypothyroidism. Maternal GD affects 0.1-0.4% of pregnancies (1–4). The fetal thyroid gland becomes responsive to thyroid-stimulating hormone (TSH) and thyrotropin receptor antibodies (TRAb) at around 20 weeks of gestation (5, 6). In mothers with GD during pregnancy, thyroid autoantibodies levels usually decrease due to immunosuppression and/or hemodilution and reach their lowest values shortly before delivery. In the postpartum period, the levels of thyroid autoantibodies recover in the mother's blood and may exceed values detected in early pregnancy (7). In pregnancy, thyroid autoantibodies freely cross the placenta and either overstimulate (thyroid stimulating antibody - TSAb) or block (thyroid blocking antibody - TBAb) the fetal thyroid gland (4, 7–9). Maternally transferred antibodies can transiently impact fetal and neonatal thyroid function until they are metabolized. A high level of TSAb transmission is associated with the occurrence of fetal and neonatal thyrotoxicosis. Maternal TBAb can induce congenital hypothyroidism (9). Thyroid function disturbances observed in the fetus/newborn depend not only on the type of maternal antibodies, but also on their levels. The antithyroid drug (ATD) administration during pregnancy and previous definitive treatment of GD (radioactive iodine [RAI] therapy or thyroidectomy) in the mother could also impact the function of the fetal/neonatal thyroid (4, 8, 10). Autoimmune hyperthyroidism also occurs in children born to mothers who were treated for GD years ago, but still have detectable circulating TRAb (4, 5, 11).

Fetal hyperthyroidism can cause goiter, heart failure with non-immune hydrops, advanced bone maturation, intrauterine growth retardation, preterm birth and even fetal death (1, 5, 6, 8). Therefore, a mother with a history of GD should be closely followed up during pregnancy. American Thyroid Association (2017) and European Thyroid Association (2018) guidelines have recommended taking measurements of maternal TRAb as soon as pregnancy is confirmed, and if elevated, repeating them at 18-22 weeks of gestation (12, 13). Neonatal autoimmune hyperthyroidism is usually transient and occurs in 1.5-2.5% of babies of GD mothers, but it is associated with an increased risk of long-term morbidity and mortality (1, 5, 14). Hyperthyroidism itself can lead to severe complications such as cardiac insufficiency, liver dysfunction, coagulopathy, craniostenosis, microcephaly and neurodevelopment disabilities (1, 14). Practice guidelines also include measurements of TRAb value in cord blood and serum levels of free thyroxine (fT4) and TSH between the 3rd and 5th day of life in neonates born to mothers with GD. Additionally, clinical observation in the first 2-3 months of life is recommended (1, 15, 16). Several studies have demonstrated that positive TRAb levels in cord blood correlate with the likelihood of development of hyperthyroidism in the first two weeks of life, whereas negative antibodies are associated with no risk of neonatal hyperthyroidism (1, 6, 14).

In fetuses/newborns of ATD treated mothers, the ATD passage across the placenta may increase the risk of the development of transient fetal/neonatal hypothyroidism (5, 15–17). It is usually observed in the first days of life until the ATD has been metabolized in the newborn's body (9, 17, 18). Some newborns born to mothers with GD may also present central hypothyroidism (CeH) due to impaired regulation of the fetal hypothalamic-pituitary-thyroid (HPT) axis (19).

The aim of our observational study was to evaluate different types of thyroid dysfunction in babies with neonatal hyperthyroidism.

MATERIALS AND METHODS

This is an observational study including children hospitalized at the Department of Paediatrics and Endocrinology of the Medical University of Warsaw, Poland, between 2014 to 2021, due to neonatal hyperthyroidism. The study was approved by the Bioethics Committee at the Medical University of Warsaw. Medical records of 14 infants (10 boys, 4 girls) were analyzed. The following maternal data were collected: the history of mother's thyroid disease, time of diagnosis (before or during pregnancy), TRAb levels and type of treatment (ATD, RAI therapy, thyroidectomy). In newborns the following data were analyzed: presence of obstetric ultrasound anomalies, gestational age, birth weight, birth length, birth head circumference, Apgar score, signs of hyperthyroidism. Birth growth parameters were reported in percentile ranks using Fenton 2013 Growth Calculator (https://peditools.org/fenton2013/) for each child. Infants were classed as small-for-gestational age (SGA) when their birth weight and/or length parameters were below the 10th percentile for gestational age (20). Time of TSH, fT4, free triiodothyronine (fT3) and TRAb levels normalization, the treatment modality was analyzed in each child.

Serum TSH (μIU/ml), fT4 (ng/dl) and fT3 (pg/ml) levels were measured by immunofluorescence method using the Architect i1000SR analyzer (Abbott Diagnostics, Abbott Park, Illinois, USA). The TRAb levels were measured by electrochemiluminescence immunoassay (ECLIA) with the Cobas e801 analyzer (Diagnostics Roche, Basel, Switzerland). Biochemical measurements were interpreted in relation to the reference range. Chosen anthropometric and biochemical data are also presented as means with standard deviation (SD) and minimum and maximum values.

RESULTS

The maternal, fetal and neonatal characteristics are presented in **Table 1**.

In our study 93% of infants (13 out of 14 cases) with neonatal hyperthyroidism were born to mothers with GD. Seven mothers were diagnosed before pregnancy (three of them were treated with ATD, two were after RAI therapy and one after thyroidectomy) and six mothers were diagnosed with GD in the first trimester of pregnancy. Only one neonate, presented as case 14, was born to a mother diagnosed five years before pregnancy with hypothyroidism in the course of Hashimoto thyroiditis and treated with levothyroxine (L-T4) until the end of

TABLE 1 | The maternal, fetal and neonatal characteristics.

	Maternal thyroid disease history				Fetus		Delivery	Neonates			
Patient	**Time of diagnosis**	**Treatment before pregnancy**	**Treatment during pregnancy**	**TRAb (IU/L) (>20 GW)**	**Symptoms of HT**	**GA (weeks)**	**Type of delivery**	**Apgar score**	**Weight gram (pc)**	**Length cm (pc)**	**Head cm (pc)**
1	GD (*4yrs)	TD, L-T4	L-T4	na	no	33	CS (FF)	10	1800 (29)	47 (92)	33 (97)
2	GD (T1)		MMI	14.8	T,G	38	CS (GDM)	10	3530 (79)	55 (>99)	34 (52)
3	GD (*3mth)	ATD	PTU	9.23	no	38	CS	10	3600 (86)	52 (92)	35 (62)
4	GD (*)?		MMI+Encorton (from 28GW)	> 40	no	33	CS (FF)	10	2190 (67)	49 (99)	31 (69)
5	GD (*10yrs)	RI, L-T4	L-T4, MMI (from 22GW)	37	no	38	CS (ophthalmopathy)	10	2960 (42)	50 (72)	34 (62)
6	GD (T1)		PTU (T1), MMI (T2,3)	11	no	39	CS (FF)	10	4000 (91)	56 (99)	35 (63)
7	GD (*6yrs)	RI, L-T4	L-T4	na	no	39	CS (previous CS)	10	3760 (80)	57 (>99)	35 (63)
8	GD (T1)		no	3.34	no	39	CS (previous CS)	10	2970 (28)	53 (93)	32 (6)
9	GD (T1)		PTU (T1), MMI (T2,3)	31.2	no	41	CS (FF)	10	3850 (55)	58 (>99)	34 (13)
10	GD (T1)		no	2.51	no	39	CS (lack of progression)	10	3310 (46)	52 (78)	35 (63)
11	GD (*3mth)	ATD	PTU (T1), L-T4 (from T2)	na	no	38	VD	8-9-10	3580 (85)	56 (100)	43 (100)
12	GD (T1)		MMI, L-T4 (from T3)	6.8	no	41	CS (lack of progression)	10	3395 (34)	57 (99)	35 (44)
13	GD (*2mth)	ATD	MMI (to 23GW)	5.11	G	39	VD	10	2925 (16)	54 (95)	34 (36)
14	HD (*5yrs)	L-T4	L-T4 (T1)	14	no	36+6/7	VD	7-8-10	2940 (52)	50 (78)	34 (70)

*GD, Graves' disease; HD, Hashimoto disease; *, diagnosis before pregnancy; yrs, years; mth, months; T, trimester; TD, thyroidectomy; L-T4, levothyroxine; ATD, antithyroid drug; RI, ^{131} Iodine therapy; MMI, methimazole; PTU, propylothiouracyl; TRAb, thyrotropin receptor antibodies; GW, gestational weeks; na, not available; HT, hyperthyroidism; T, tachycardia; G, goiter; GA, gestational age; CS, caesarean section; VD, vaginal delivery; GDM, gestational diabetes mellitus; FF, fetal factors; pc, percentile; cm, centimeter.*

Cut-off point for positive TRAb: >1.75 IU/L.

the first trimester of pregnancy. The treatment was withdrawn because of TSH inhibition. Positive TRAb levels were detected in her after 20 weeks of gestation. From the second trimester she did not need L-T4 administration until the end of pregnancy and after delivery. In three cases maternal TRAb levels were not available for analysis, in all the other cases TRAb levels evaluated after 20 weeks of gestation were positive (ranging from 2.51 to more than 40 IU/L). Only in two cases (14%) the presence of fetal goiter and tachycardia was confirmed using ultrasound scans during pregnancy (cases 2 and 13). Majority of newborns (85%) were born in time; two babies were born preterm at 33rd week of gestation. All the babies were born in a good condition, none of them was born as SGA. In 79% of cases (11 out of 14) the delivery was by caesarean section.

Groups of Patients Divided According to Thyroid Dysfunction After Birth

Characteristics of thyroid function and the type of therapy in all the studied babies are presented in **Table 2**.

1 Overt Hyperthyroidism

Six out of the 14 babies (43%, cases: 1-6) showed overt hyperthyroidism with highly increased TRAb levels (mean 13.0 ± 7.0 IU/L, range: 6.26 - 21.2 IU/L). Maternal TRAb levels measured after 20 weeks of gestation were also high (mean 22.4 ± 14.8 IU/L, from 9.23 to more than 40 IU/L). All those babies required ATD treatment, which was started between 5 to 9 days of life with an initial daily dose of around 0.5 mg/kg. Mean duration of ATD treatment was 22 days (range: 13 - 46 days). Three babies in this group required further L-T4 supplementation (cases 4 and 6 subsequently to MMI therapy and case 1 one month after the end of MMI treatment). In two of them central hypothyroidism was diagnosed (cases 1 and 4) and in one primary hypothyroidism (case 6). One neonate (case 1) was born prematurely in the 33rd week of pregnancy. Only one child in these group (case 2) presented goiter and tachycardia in fetal life. Tachycardia was observed after the birth in all that babies.

2 Hyperthyroidism With Low Normal TSH Level

Six out of 14 children (43%, cases: 7-10,12,14) presented hyperthyroidism with slightly increased fT4 and/or fT3 levels and TSH levels in the lower limit of the normal range coinciding with positive TRAb serum levels, but not exceed 6 IU/L (mean 3.8 ± 1.6 IU/L, range: 2.24 - 5.76 IU/L). Three of these babies due to tachycardia received only propranolol therapy for 2 to 46 days with an initial dose of 0.5 mg/kg. Two other children (cases 9 and 14) were treated for 29 and 18 days with initial dose of MMI 0.25 and 0.4 mg/kg, respectively. The neonate born to a mother diagnosed with Hashimoto thyroiditis before pregnancy (case 14) also demonstrated transient tachycardia. One neonate (case 12) presented a slightly elevated fT4 level with a normal TSH level in the third day of life, which did not require any pharmacological therapy. However, in the following few weeks decrease in TSH levels below the lower limit of the range and low normal fT4 levels were found and L-T4 supplementation was administered for seven months.

TABLE 2 | Characteristics of thyroid function and the type of therapy in all the studied babies.

	First thyroid blood test (start of treatment)					Thyroid blood test (end of MMI treatment)					Treatment		Follow-up	
Patient	Age	TSH µIU/ml	fT4 ng/dl (pmol/l*)	fT3 pg/ml (pmol/l*)	TRAB IU/L	Age	TSH µIU/ml	fT4 ng/dl (pmol/l*)	fT3 pg/ml	TRAB IU/L	Treatment duration (d)	IDD mg/kg b.w	Observation period	Treatment follow-up
1	6 d	**0.00**	**76.03*** (9-21)	**29.52*** (3.8-6.0)	na	28 d	**0.00**	1.33	4.0	**5.27**	MMI (6-28)	0.5	7 yrs	LT4 (2/12-6.5 yrs)
2	3/9 d	**0.138/0.02**	**78.59*** (13.9-26.1)/**3.6**	**14.61*** (3-8.1)/**11.04**	**6.26**	22 d	5.97	0.82	4.13	na	MMI (9-22)	0.5	3.5/12	–
3	3/5 d	**0.02/0.09**	**27.05*/38.5*** (10.94-23.68)	na/**18.21*** (3.44-7.59)	na	22 d	**0.28**	0.93	4.71	**2.21**	MMI (5-22)	0.5	3/12	–
4	5/7 d	**0.051/0.005**	1.76/**3.18**	**5.62/7.29**	**21.2**	30 d	1.27	**<0.42**	1.76	**8.05**	MMI (7-30)	0.6	9/11	LT4 (1/12-na)
5	7 d	**<0.01**	**2.36**	4.84	**16.4**	51 d	**0.51**	0.87	3.19	**3.23**	MMI (5-51)	0.3	2/12	–
6	3/7 d	2.588/**0.373**	**34.48*/34.96*** (9-52- 30.8)	4.99*/na* (3.44-7.59)	**8.16/7.36**	22 d	**19.93**	**8.71*** (11.1-27.3)	2.66	**3.93**	MMI (7-22)	0.5	9/12	LT4 (22d-continue)
7	4 d	2.143	**2.06**	na	**2.43**	14 d	na	na	na	0.96	Propranolol (4-14)	0.5	3/12	–
8	3 d	2.34	**2.67**	**6.18**	**4.76**	51 d	na	na	na	**1.85**	Propranolol (5-51)	0.5	4/12	–
9	5 d	1.74	**2.54**	na	**5.27**	34 d	9.26	**0.77**	3.38	**1.76**	MMI (5-34)	0.25	3/12	–
10	2/5 d	19.06/**1.1**	1.42/1.74	**5.68**/2.93	**2.3**	15 d	1.67	1.24	3.87	**2.3**	Propranolol (2-4)	0.5	15 d	–
11	2 d	**0.99**	1.17	2.74	< 0.8 CB	42 d	6.24	13.05* (10.29-21.88)	na	na	observation	–	1.5/12	–
12	3 d	6.54	**1.98**	na	**2.24** CB	3/12	**0.85**	1.02	3.98	na	observation	–	10/12	LT4 (<3/12-10/12)
13	25 d	**0.5**	1.22	4.09	**1.92**	3.5/12	**0.75**	1.12	3.72	1.34	observation	–	6.5/12	–
14	2 d	1.76	**55.67*** (13.9-26.1)	na	**5.76**	20 d	5.91	1.15	3.78	na	MMI (2-20)	0.4	6/12	–

TSH, thyroid-stimulating hormone; fT3, free triiodothyronine; fT4, free thyroxine; TRAb, thyrotropin receptor antibodies; MMI, methimazole; LT4, levothyroxine; d, day of life; yrs, years; na, not available; CB, cord blood; IDD, initial daily dose; b.w, body weight; Normal values: TSH - 0-3 days: 1.3-19.0 µIU/ml, 3-70 days: 0.6-17.0 µIU/ml, 70-356 days: 0.88-5.42 µIU/ml; fT4 - 0-3 days: 0.80-1.90 ng/dl, 3-70 days: 0.80- 1.70 ng/dl, 70-356 days: 0.89-1.72 ng/dl; fT3 - 0-12/12: 2.24-4.94 pg/ml; normal values of fT4 and fT3 in pmol/l are enclosed in bracket, next to the result; Cut-off point for positive TRAb: >1.75 IU/L.

3 Isolated TSH Suppression

Normal thyroid hormone levels with TSH levels below the lower limit of the range were observed in the neonates presented as cases 11 and 13. Those two babies did not require pharmacological therapy. In one of them (case 13) low TSH levels maintained until the fourth month of life and next spontaneously normalized. Maternal TRAb levels in those cases were 3.6 IU/L before pregnancy (not evaluated during pregnancy) in case 11 and 5.11 IU/L during pregnancy in case 13.

DISCUSSION

This study analyzed the thyroid function disturbances in infants diagnosed with hyperthyroidism in the neonatal period. As expected, almost all babies were born to mothers with GD, except for one child who was born to a mother with Hashimoto thyroiditis treated with L-T4. The presence of TRAb is rarely documented in previously hypothyroid individuals. In the above-mentioned case, after the first trimester of pregnancy the L-T4 supplementation had to be withdrawn because of TSH inhibition, but the mother in question did not develop overt hyperthyroidism and did not need to be treated with ATD. She remained euthyroid until the end of pregnancy and after delivery. A similar case was described by Kiefer et al. (21). The authors suggested that this phenomenon of unique coincidence of TSAb-induced fetal hyperthyroidism and maternal hypothyroidism results from the disappearance of the stimulating effect of TSAb on the mother's thyroid because of severe chronic autoimmune-induced damage of thyroid tissue (21).

Typically, the presence of TRAb in the mother can lead to the development of autoimmune hyperthyroidism in the fetus and neonate (17, 22). It is recommended that the fetus should be closely monitored throughout pregnancy when maternal TRAb values exceed 5 IU/L or if TRAb levels are 3 times higher than the upper limit of the normal range (12, 13). The study by Gietka-Czernel et al. (4) showed that fetal goiter is the earliest and most characteristic sign of hyperthyroidism. Tachycardia and advanced bone age occur later. In the present study, the presence of fetal goiter was documented in two babies with maternal TRAb levels of 14.8 IU/L and 5.11 IU/L, respectively. Surprisingly, one child (case 4), that of the mother with TRAb levels higher than 40 IU/L during pregnancy, did not have any symptoms of thyrotoxicosis. This baby was born preterm and developed overt hyperthyroidism, followed by CeH diagnosed soon after withdrawal of MMI therapy. His mother had poorly controlled GD with thyroid crisis in the 28th week of gestation. We suppose that CeH in this child resulted from the coincidence of high dose of MMI, devastating biosynthesis of thyroid hormones in the fetus, and the impact of high TRAb levels on the ultra-short loop pituitary feedback mechanism during fetal development.

In order to reach fetal euthyroid status when the mother is treated with ATD, the European Thyroid Association and American Thyroid Association guidelines recommend the use in mothers of the lowest possible effective ATD dose which maintains serum fT4 levels at or slightly above the upper limit of the pregnancy-specific ranges (12, 13). Iwaki et al. (23) investigated the dose-dependent effect of ATD on both maternal and fetal thyroid hormone status and confirmed a dose-dependent influence of ATD on the difference in serum fT4 levels between mothers treated with high propylthiouracil dosage (>100 mg daily) or MMI dosage (>5 mg daily) and their neonates, who had significantly lower cord blood fT4 levels than maternal serum fT4.

Our study indicates that tachycardia could be the sole clinical manifestation of hyperthyroidism in a newborn. Other symptoms, such as poor weight gain despite good appetite, irritability, hypertension, tachypnoe and ocular protrusion, have been reported in other studies (11, 24, 25).

Our observations confirmed that the types of thyroid dysfunctions in neonates are mainly determined by the TRAb level in the mother during pregnancy. The multicenter study by Banigé et al. (17) indicates that the optimal cut-off value of maternal TRAb is 2.5 IU/L for predicting fetal thyroid hypertrophy and 5.9 IU/L for predicting neonatal thyroid dysfunction. When using the neonatal TRAb level, measured in cord blood at delivery or in peripheral blood between 0 and 5 days of life, the recommended cut-off value for predicting thyroid dysfunction in a neonate is 6.8 IU/L with a sensitivity of 100% and a specificity of 94% (17). Our observations are in line with the above-mentioned results except for the risk of thyroid hypertrophy. The coincidence of high maternal TRAb levels with normal thyroid function in a neonate born to a mother with a history of GD is rarely described (4, 18, 26). It can be explained by the balance of simultaneously maternally transmitted TSAb and TBAb levels in the neonate's circulation. An analysis by Benlarbi et al. (26) shows that the majority of neonates with TRAb levels above 6 IU/L develop transient hyperthyroidism, 15% are diagnosed with primary hypothyroidism or CeH, and only 12% remain euthyroid. In addition, it has been confirmed that babies born to mothers after definitive treatment of GD before pregnancy are also at risk of hyperthyroidism at birth. Shortly after RAI therapy, TRAb levels increase. The study by Yoshihara et al. (10) strictly indicates that the risk of autoimmune hyperthyroidism in newborns is inversely related to the time lapse after RAI therapy in the mother. On the other hand, in women who underwent RAI therapy several years before pregnancy and have only slightly elevated TRAb levels in early pregnancy, TRAb levels could rise to high values at delivery (27). In our study, two mothers underwent RAI therapy three years before pregnancy. One of them developed severe thyrotoxicosis with high TRAb levels (37 IU/L) and ophthalmopathy during pregnancy. The risk of fetal/neonatal hyperthyroidism is lower in babies born to women with GD treated with thyroidectomy before pregnancy, but TRAb levels should also be assessed in them early in pregnancy and at 18-22 weeks of gestation (12, 13).

Neonatal hyperthyroidism is not the only consequence of GD in the mother. The non-obvious consequence of maternal GD leading to prolonged CeH should also be taken into account. It has been confirmed that increased transplacental passage of maternal thyroid hormones may disturb physiologic

maturation and regulation of the fetal HPT axis during intrauterine life (19). Excessive fetal thyroid hormones production in response to stimulation by maternal TRAb may also diminish fetal TSH secretion. Overexposure to thyroid hormones might alter the fetal pituitary TSH secretion set point (28, 29). Animal studies have shown that increased levels of thyroid hormones *in utero* could decrease the number of fetal pituitary thyrotrophs and TSH receptors (30, 31). Neonatal pituitary hyporesponsiveness to thyrotropin-releasing hormone (TRH) stimulation and to fT4 levels is also well-documented (29, 32). CeH is usually transient and appears at birth or follows transient neonatal thyrotoxicosis after a decrease in TSAb activity (33). The return of the HPT axis to normal function usually takes from 3 to 19 months, but in some cases as long as 3.5 years (1, 28, 29, 32). In our group in one patient the process lasted as long as 6.5 years. The much longer persistence of CeH than that of detectable TRAb levels suggests that it is related not only to the effect of TRAb on the ultra-short loop axis, but it is also associated with marked HPT axis impairment including receptors sensitivity and gene expression during fetal life. Disclosure of CeH could be delayed due to its masking by transient hyperthyroidism observed in the first weeks of life, therefore there is a need for prolonged monitoring of thyroid function in the offspring of mothers with GD.

The main limitation of the present analysis is the small size of the study group. On the other hand, we analyzed a selected group of children with thyroid dysfunction, i.e., only those patients who required hospitalization.

CONCLUSION

Our study highlights the need for prolonged monitoring of thyroid function in children born to mothers with GD. Transient hyperthyroidism is the main thyroid dysfunction in that group of children, but primary or central hypothyroidism requiring L-T4 supplementation could appear at birth or after withdrawal of ATD treatment. Diagnosis of CeH could be delayed due to its masking by transient hyperthyroidism. Prolonged TSH suppression after TRAb elimination should be considered as a signal announcing CeH.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Medical University of Warsaw. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

BP analyzed maternal and infants data, wrote the manuscript, and collected the literature data. MR designed the study, recorded and analyzed maternal and infants data, wrote the manuscript and prepared tables, collected the literature data. EW-S designed the study, recorded and analyzed maternal and infants data, wrote the manuscript, collected the literature data. AK recorded and analyzed maternal and infants data and wrote the manuscript. All authors contributed to the article and approved the submitted version.

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Correlation Between Thyroid Hormone Concentrations and Ultrasound Thyroid Volume in Preterm Infants Born Before 33 Weeks of Gestation

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Objective: Thyroid disorders are commonly concomitant with premature birth; however, indications to start therapy remain unclear due to a lack of gestational age (GA)-specific reference ranges. We aimed to evaluate the age-specific thyroid-stimulating hormone (TSH), free thyroxine (FT4) levels and the correlation between TSH and FT4 serum levels and ultrasound thyroid volume in preterm infants.

Materials and Methods: This was an observational, prospective, single-center study of 98 preterm infants born before 33 weeks GA. The infants were divided into the 24-28 weeks and 29-32 weeks GA groups. TSH and FT4 serum levels were measured at two time points: at postnatal age (PNA) 2 weeks and at postmenstrual age (PMA) 32 weeks; the results were compared between groups at two consecutive time points.

Results: There was a statistically significant between-group difference in FT4 concentration. There was a positive correlation between FT4 and GA at both screening times. FT4 in the 24-28 weeks GA group was significantly lower than in the 29-32 weeks GA group. The mean (standard deviation [SD]) FT4 at PNA 2 weeks was 11.72 ± 2.16 pmol/l for the 24-28 weeks GA group vs. 13.33 ± 1.80 pmol/l for the 29-32 weeks GA group ($p<0.001$). The mean (SD) FT4 at PMA 32 weeks was 11.96 ± 1.98 pmo/l for the 24-28 weeks GA group vs. 13.33 ± 1.80 pmol/l for the 29-32 weeks GA group ($p=0.001$). Our results reflect a slow and gradual upward trend of FT4 in the 24-28 weeks GA. It is of interest that the correlation between thyroid volume and FT4 was statistically significant (rho=0.25, $p=0.019$) for all studied preterm infants. The correlation between thyroid volume and weight was statistically significant for the entire study group (rho=0.37, $p<0.001$). We did not find statistically significant differences in TSH and FT4 values between consecutive time points at 24-28 weeks GA. The thyroid volume was not significantly different between both groups. The total thyroid volume was 0.26 vs. 0.27 ml for the 24-28 and 29-32 weeks GA groups, respectively.

Conclusion: The results of this study indicate that preterm infants require lower FT4 values depending on GA. Moreover, ultrasound thyroid imaging may facilitate the evaluation of questionable thyroid disorders.

Keywords: thyrotropin, thyroxine, preterm infants, ultrasound, thyroid volume

INTRODUCTION

Thyroid hormones are crucial for metabolism and thermogenesis and thus for the normal development of the central nervous system in fetuses and infants. Congenital hypothyroidism (CH), the most common endocrinological disorder, is a preventable cause of neurodevelopmental disability. Early diagnosis and treatment of CH can help prevent neurodevelopmental impairment and improve long-term outcomes (1).

The newborn screening test (NST) for CH has recently been improved through the adoption of a lowered thyroid-stimulating hormone (TSH) level as a basis for the diagnosis of CH, is one of the most valuable tools for preventing intellectual disability (2). The increasing incidence of positive screening tests for hypothyroidism performed in all infants, including preterm infants, has given new, greater importance to the issue.

Thyroid disorders are commonly associated with prematurity (3, 4). The higher incidence of infant hypothyroidism might be associated with an increased survival rate of preterm neonates, along with changes in the NST. The prevalence of transient hypothyroidism in preterm infants below 1500 g at birth is 14-fold higher than that in mature infants (4). Preterm infants are more vulnerable to thyroid dysfunction for many reasons, including hypothalamic-pituitary immaturity, serious neonatal illness, impaired synthesis and metabolism of thyroid hormones, and the use of drugs (e.g., dopamine or steroids) (2–6).

The thyroid response to the action of drugs administered to preterm infants has not been sufficiently studied. Dopamine, an adrenergic neurotransmitter that inhibits TSH secretion, also suppresses thyroxine (T4) secretion. However, the discontinuation of dopamine administration results in an immediate increase in T4 levels (4, 7, 8). In contrast to dopamine, dobutamine does not influence the thyroid hormone profile (8). Steroid administration can diminish the TSH response and peripheral conversion of T4 to T3 (4, 7). Studies conducted by Arai et al. confirmed a rapid elevation of free thyroxine (FT4) serum levels in samples collected two days after steroid withdrawal (7).

However, thyroid disorders in preterm infants remain unrecognized. This is likely due to a delay in the elevation of TSH concentration after delivery (1, 5). TSH concentrations tend to rise between the second and sixth weeks of life, although the exact time of TSH elevation can vary. To cope with the resultant problem, European and American guidelines recommend a repeated screening test for hypothyroidism in preterm infants. According to European guidelines, rescreening for CH should be performed in all preterm infants two weeks after the first screening or in the second week of life. The American Academy of Pediatrics advocates repetition between the second and sixth weeks of life (4). However, there is no universal agreement on the optimal timing of blood sample collection for hypothyroidism assessment in preterm infants. Consequently, novel methods for assessing thyroid function are being sought. Thyroid ultrasound imaging might perform this function.

Ultrasound might prove helpful in determining the anatomy and function of the thyroid gland in infants (9). In particular, ultrasound-assessed thyroid volume in neonates may help detect thyroid dysfunction. Ultrasound examination also provides information on the location and structure of the gland. Although ultrasonography helps reveal abnormalities in the location of the thyroid gland, it is not sufficient to diagnose its hypertrophy or hypoplasia (10). Given the simplicity, non-invasive character, and repeatability of the ultrasound examination, we strongly advocate that it should be included as a standard examination in the initial diagnosis of neonates with abnormal screening test results for hypothyroidism. Our opinion is consistent with the guidelines on CH from the European Society for Pediatric Endocrinology, which recommends the performance of imaging studies to determine a specific etiology (11).

There are no currently unavailable normative thyroid ultrasound data for neonates, although significant differences in the mean thyroid volume were detected on ultrasound according to gestational age (GA) (12). The determination of normative ultrasound thyroid volume values could allow the objective identification of a gland as normal, small, or enlarged. Combined with age-specific thyroid hormone reference levels, these reference values could be used to help neonatologists interpret thyroid hormone and ultrasound results in neonates with greater accuracy.

This study aimed to determine the specific GA TSH and FT4 levels in preterm infants born at <32 weeks GA and determine the distribution of FT4 values. Therefore, the preterm infants in our study were divided into two groups: those born between 24-28 and 29-32 weeks GA. In addition, we sought additional methods to assess the physiology of the development and dysfunction of the thyroid gland in preterm infants. We also attempted to determine whether the concentrations of thyrotropic hormone and FT4 correlate with the ultrasound thyroid volume and GA, thus decreasing the percentage of false-negative cases. We further attempted to determine the optimal chronological age that correlates with delayed TSH elevation.

SUBJECTS AND METHODS

Subjects and Eligibility Criteria

This was an observational, prospective, single-center, population-based cohort study. Preterm infants born between 24 and 32 weeks of gestation who were born in or transferred to

the Neonatal and Intensive Care Department of the Medical University of Warsaw in Princess Anna Mazowiecka Hospital (Poland) were recruited for this study. The patients' GA was estimated using ultrasonography. The study was initiated in January 2020, and patients were recruited between 2020 and 2021. Ninety-eight participants were recruited within 7 days of birth.

The inclusion flowchart is presented in **Figure 1**. The eligibility of prospective patients was determined by recruiting physicians familiar with the study protocol. Subjects with the following conditions were excluded from the study: preterm delivery <23 or >32 weeks GA; major congenital abnormalities; administration of medications such as steroids or vasopressors such as dopamine (up to 12 hours after the end of treatment), positive maternal thyrotropin antibodies, thyroid disease of mothers treated with antithyroid drugs or amiodarone, and lack of parental consent.

Clinical Variable Collection

Maternal medical history included antenatal steroids, maternal diabetes mellitus, and thyroid disorders. The perinatal data included GA, body weight at birth, sex, z-score of birth weight, 1- and 5-min Apgar scores, and delivery method. Clinical data, such as sepsis, necrotizing enterocolitis (NEC), retinopathy of prematurity (ROP), postnatal steroid therapy, dopamine administration, and invasive or non-invasive ventilation, were obtained at discharge. Thyroid function data, including postnatal age (PNA), serum TSH concentration, serum FT4 concentration, and ultrasound thyroid volumes of all recruited preterm infants, were collected prospectively.

To avoid a surge in TSH after birth, blood samples were collected at PNA 2 weeks, and the blood TSH and FT4 levels were measured at 32 postmenstrual age (PMA). The ultrasound thyroid volume was performed at PMA 32 weeks. The baseline data were collected in the hospital electronic database.

Grouping of the Subjects

The study included 98 infants divided into two groups: those born at 24–28 weeks GA and those born at 29–32 weeks GA. In the 24–28 weeks GA group, FT4 and TSH concentrations were evaluated at 14–21 days of life and PMA 32 weeks. In the 29–32 weeks GA group, FT4 and TSH concentrations were measured at PMA 32 weeks (after 14-21 days of life, equivalent to PMA 32 weeks).

Detection Methods

Blood samples (1 ml) were collected and examined in the hospital laboratory to measure the levels of serum thyroid hormones

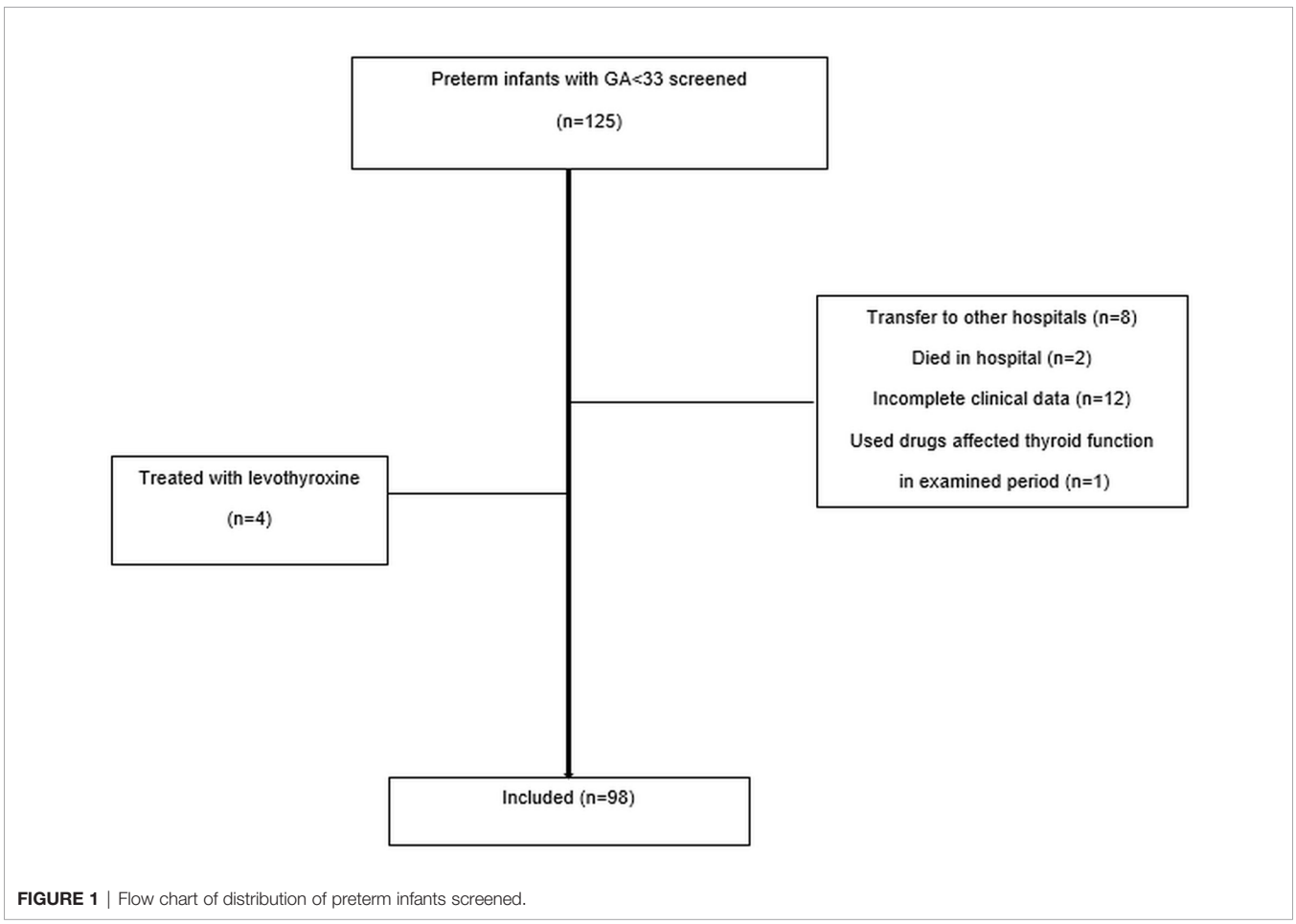

FIGURE 1 | Flow chart of distribution of preterm infants screened.

during other routine blood tests. The immunofluorescence assays of Architect i1000SR of the Abbott Diagnostic test were used to detect TSH and FT4 levels in serum samples.

Ultrasound evaluation of the neonate thyroid was performed using a Philips HD XE system. (Philips Healthcare, Eindhoven, the Netherlands). One observer scanned the babies at the bedside using a portable scanner. The thyroid gland was measured using a linear array transducer with a high-frequency probe (L7-15 MHz). The volume of the thyroid gland was calculated using the formula for a prolate ellipsoid, where thyroid volume = length × breadth × depth × π/6 (0.52). The total thyroid volume was calculated as the sum of the volume of the individual lobes, disregarding the volume of the isthmus, since this is very low in normal neonates (13).

Primary Outcomes

The primary outcomes were as follows: (a) assessment of the thyroid function and volume depending on the gestation age; (b) the determination of FT4 and TSH values at 14–21 days of life and at PMA 32 weeks in preterm infants; (c) the determination of the ultrasound thyroid volume at PMA 32 weeks in preterm infants; and (d) the evaluation of correlations between circulating thyroid hormone concentrations and thyroid volume.

Secondary Outcomes

The secondary outcomes were as follows: (a) the comparison of changes in FT4 evaluated at 14-21 days of life (PNA 2 weeks) and PMA 32 weeks in the 24-28 weeks GA group; (b) the comparison of changes in TSH evaluated at 14-21 days of life (PNA 2 weeks) and PMA 32 weeks in the 24-28 weeks GA group; (c) the comparison of changes in FT4 and TSH evaluated at 14-21 days of life (PNA 2 weeks) between the 24-28 and 29-32 weeks GA groups; (d) the analysis of TSH values over time (to determine the optimal time for TSH measurement); (e) the evaluation of the correlation between thyroid volume and circulating thyroid hormone concentrations with body mass at PMA 32 weeks; and (f) the assessment of the influence of morbidity and drugs administered to neonates on thyroid volume and function.

Ethical Approval

The study was registered in the Clinical Trials Registry (Registration No. NCT04208503; protocol version 07.01.2020) and approved by the Bioethical Committee of the Medical University of Warsaw (KB44/2019). The participants' caregivers were informed about the study's methods and purpose. Written informed consent was obtained from all the participants.

Statistical Analysis

Statistical analysis was carried out using the R package, version 4.0.5. Nominal variables are presented as n (%), continuous variables as means ± standard deviation (SD), or median (quartile [Q]1, Q3), depending on the data distribution. The data's normality was validated using the Shapiro-Wilk test and based on skewness and kurtosis values. Groups were compared using the chi-square test or Fisher's exact test for nominal variables and with Welsch t-test or Mann–Whitney U test for continuous variables, as appropriate. The means and median differences (MD) were calculated, including the 95% confidence interval (CI). Paired comparisons were made using the paired t-test or Wilcoxon test. The correlation analysis was performed using Spearman's correlation coefficient. Additionally, the agreement between double measurements of thyroid volume for the 32nd week was assessed using the interclass correlation coefficient.

RESULTS

A total of 125 preterm infants were screened. The final sample included 98 preterm infants after the exclusion of four subjects due to the diagnosis of thyroid disorders, the death of two in the postnatal period, the transfer of eight to another hospital, and one due to the administration of drugs that affected thyroid function, and 12 due to incomplete clinical data. Nine patients were small for GA (SGA), and 26 were born from multiple pregnancies. Our analysis included 37 males (37.8%) and 61 females (62.2%). The mean (SD) GA was 28.26(± 2.29) weeks, and the mean birth weight (BW) was 1226.02(± 381.57) g. Among the 98 preterm infants, there were 49 (16 male, 33 female) in the 24-28 weeks GA group and 49 (21 male; 28 female) in the 29-32 weeks GA group. The overall group characteristics are presented in **Table 1**.

There were statistically significant between-group differences regarding GA, BW, 1- and 5-min Apgar scores, postnatal steroid and dopamine therapy use, mechanical ventilation frequency, and antibiotics administration ($p<0.001$). We did not identify statistically significant between-group differences regarding the frequency of CPAP(continuous positive airway pressure), NEC, ROP, and prenatal steroid use.

There was a statistically significant difference in FT4 concentration between the groups. The FT4 in the 24-28 weeks GA group was significantly lower than in the 29-32 weeks GA group. The mean (SD) FT4 at PNA 2 weeks was 11.72 ± 2.16 pmol/l for the 24-28 weeks GA group vs. 13.33 ± 1.80 pmol/l for the 29-32 weeks GA group, MD = -1.61, 95% CI [-2.49, -0.74], $p<0.001$; and the mean (SD) FT4 at PMA 32 weeks was 11.96 ± 1.98 pmo/l for the 24-28 weeks GA group vs. 13.33 ± 1.80 pmol/l for the 29-32 weeks GA group, MD=-1.37, 95% CI [-2.13, 0.61], $p=0.001$.

No statistically significant between-group differences were found in the TSH values at PNA 2 weeks and PMA 32 weeks. The median (Q1, Q3) TSH at PNA 2 weeks was 2.55 (1.53, 4.36) mU/l for the 24-28 weeks GA group vs. 2.42 (1.45, 3.22) mU/l for the 29-32 weeks GA group, $p=0.650$; at PMA 32 weeks the median (Q1, Q3) TSH was 2.55 (1.53, 4.36) mU/l for the 24-28 weeks GA group and 2.42 (1.45, 3.22) mU/l for the 29-32 weeks GA group, $p=0.457$ (**Table 2**).

Thyroid volume was not significantly different between the two groups. The median of total thyroid volume performed at PMA 32 weeks for the 24-28 weeks GA group and for the 29-32weeks GA group was 0.26 (0.22, 0.31) and 0.27 (0.24, 0.34) ml, respectively; $p=0.318$ (**Table 2**). There were no statistically significant differences between the two consecutive screening tests for FT4 and TSH in the 24-28 weeks GA group (**Table 3**).

TABLE 1 | Demographic characteristics of the study cohort.

	24-28 weeks GA	29-32 weeks GA	p-value
n	49	49	
Sex, female, n (%)	33 (67.3)	28 (57.1)	NS
Model of delivery, n (%)			
Caesarean section	31 (63.3)	33 (67.3)	NS
Natural delivery	18 (36.7)	16 (32.7)	NS
GA	26.33 ± 1.39	30.18 ± 1.03	<0.001
SGA/AGA/LGA preterm infants, n (%)			
SGA	4 (8.2)	5 (10.2)	NS
AGA	40 (81.6)	41(83.7)	NS
LGA	5 (10.2)	3 (6.1)	NS
Birth weight, g, mean ± SD	954.69 ± 222.39	1 497.35 ± 307.35	<0.001
Birth weight at 32PMA, g, mean ± SD	1 419.57 ± 235.14	1 556.73 ± 281.49	0.010
Apgar score, 1 min, median (Q1;Q3)	6.00 (5.00;7.00)	7.00 (6.00;8.00)	0.002
Apgar score 5 min, median (Q1;Q3)	7.00 (7.00;8.00)	8.00 (7.00;9.00)	<0.001
Singleton or multiplets, n (%)			
Singleton	41 (83.7)	32 (65.3)	NS
Multiplets	8 (16.3)	17 (34.7)	NS
Prenatal Steroid, n (%)	39 (79.6)	34 (69.4)	NS
Postnatal Steroid, n (%)	12 (24.5)	0 (0.0)	<0.001
Dopamine therapy, n (%)	7 (14.3)	0 (0.0)	0.012
CPAP, n (%)	49 (100.0)	46 (93.9)	NS
Mechanical ventilation, n (%)	35 (71.4)	16 (32.7)	<0.001
Necrotizing enterocolitis, n (%)	0 (0.0)	2 (4.1)	NS
Neonatal Sepsis, n (%)	6 (12.2)	1 (2.0)	NS
ROP, n (%)	4 (8.2)	0 (0.0)	NS
Mothers treated with levothyroxine, n (%)	9 (18.4)	11 (22.4)	NS
Antibiotics, n (%)	18 (36.7)	0 (0.0)	<0.001

AGA, appropriate for gestational age; CPAP, continuous positive pressure; LGA, large for gestational age. Groups were compared using the chi-square test or Fisher's exact test for nominal variables and the t-test or Mann–Whitney U test for continuous variables; NS, non-statistical.

TABLE 2 | Thyroid parameters between groups categorized by GA.

	24-28 weeks GA	29-32 weeks GA	MD (95% CI)	p-value
TSH – PMA 32 weeks	2.13 (1.41; 3.12)	2.42 (1.45; 3.22)	-0.29 (-0.82; 0.38)	NS
FT4 – PMA 32 weeks	11.96 ± 1.98	13.33 ± 1.80	-1.37 (-2.13; -0.61)	0.001
TSH – PNA 2 weeks	2.55 (1.53; 4.36)	2.42 (1.45; 3.22)	0.13 (-0.59; 0.93)	NS
FT4 – PNA 2 weeks	11.72 ± 2.16	13.33 ± 1.80	-1.61 (-2.49; -0.74)	<0.001
Volume L – PMA 32 weeks	0.14 (0.11; 0.16)	0.13 (0.11; 0.17)	0.01 (-0.02; 0.02)	NS
Volume R – PMA 32 weeks	0.13 (0.11; 0.15)	0.14 (0.12; 0.19)	-0.01 (-0.03;0.01)	NS
Difference (R minus L)	-0.01 (-0.02; 0.01)	0.00 (-0.02; 0.02)	-0.01 (-0.02;0.002)	NS
Total Thyroid volume-PMA 32 weeks	0.26 (0.22; 0.31)	0.27 (0.24; 0.34)	-0.01 (-0.05;0.02)	NS

TG, thyroid gland; R, right; L, left. Data are presented as median (Q1, Q3) or mean ± SD. Groups were compared using the t-test or Mann–Whitney U test. NS, non-statistical.

The correlation between thyroid volume, FT4 and TSH concentration level, weight, and GA were assessed in all studied preterm infants in the 24-28 and 29-32 weeks GA groups. The correlation between the thyroid volume and weight was statistically significant for the whole study group (rho=0.37, p<0.001) as well as for the 29-32 weeks GA group (rho = 0.55, p < 0.001). Overall, the correlation between thyroid volume and FT4 was statistically significant (rho = 0.25, p = 0.019) for all studied preterm infants at a PNA of 2 weeks. Similarly, there was a positive correlation between FT4 and GA at both screening times at a PNA of 2 weeks (rho = 0.42, p<0.001) and at PMA 32 weeks (rho=0.35, p<0.001). No statistically significant correlation was found for the 24-28 weeks GA group between the thyroid volume and GA, nor for FT4 and TSH. There was an inverse correlation between thyroid volume and TSH concentration level in the 29-32 weeks GA group (rho=-0.34, p=0.018).

These results indicate that factors such as the out-of-the-schedule-blood-test-time administration of postnatal steroids and dopamine did not significantly affect the thyroid parameters in any way in the 24-28 weeks GA group. They were not affected by sepsis, ROP, or NEC. The intra-observer variation was calculated based on 20 sonographic measurements to be 5.6 ± 19.2%, MD=0,87, 95% CI [0.70-0.95].

DISCUSSION

Preterm infants have a higher risk of thyroid dysfunction than mature infants. In particular, preterm infants show an atypical form

TABLE 3 | Comparison of TSH and FT4 according to PNA 2 weeks and PMA 32 weeks in the 24-28 weeks GA group.

	PNA 2 weeks	PMA 32 weeks	MD (95% CI)	p-value
n	49	49		
TSH (mU/l)	2.55 (1.53; 4.36)	2.13 (1.41; 3.12)	-0.42 (-1.23; 0.21)	0.218
FT4 (pmol/l)	11.72 ± 2.16	11.96 ± 1.98	0.24 (-0.65; 1.43)	0.432

Data are presented as median (Q1, Q3) or mean ± SD. The groups were compared using the t-test or Mann–Whitney U test.

TABLE 4 | Distribution of FT4 (pmol/l) values in preterm infants born <33 weeks GA, measured at 2 weeks PNA.

GA (weeks)	5p	10p	25p	50p	75p	90p	95p
24-26 (n=19)	8.129	8.772	9.73	11.11	13.1	14.248	14.951
27-28 (n=20)	8.211	8.856	11.426	12.12	12.855	13.336	15.003
29-30 (n=33)	10.898	11.068	12.2	13.05	13.97	14.73	15.148
31-32 (n=16)	10.988	11.315	12.325	13.64	15.315	17.15	18.2425

p, percentile.

of hypothyroidism, which presents a challenge in distinguishing cases of CH and interpreting NST (1, 2, 14). Moreover, there are no reliable standard thyroid hormone reference values for preterm infants. Hence, neonatologists are increasingly aware of the challenging importance of diagnosing and treating thyroid dysfunction in preterm infants. This study investigated the association between thyroid hormone levels and thyroid volume in preterm infants and allowed us to obtain FT4 and TSH concentration values in preterm infants born before 33 weeks GA and evaluate the ultrasound thyroid volume at PMA 32 weeks.

Thyroid disorders in preterm infants include a unique form of hypothyroidism characterized by transient hypothyroxinemia of prematurity (THOP) or delayed TSH elevation and hyperthyrotropinemia despite low serum FT4/T4 (1, 2, 15). THOP is defined as a temporary reduction in FT4 values due to a blunted postnatal increase with no increase in TSH values, which may last from six to eight weeks (4). Hypothyroxinemia of prematurity is a well-described condition. Its incidence in preterm infants varies between 35% and 85% (15, 16). The reported wide discrepancy in the frequency of THOP diagnosis may reflect the lack of consensus about the FT4 level that constitutes the "low" for that specific GA in preterm infants.

The first finding of our study was that the FT4 serum level positively correlated with GA in the whole study group (rho=0.42; p<0,001 at PNA 2 weeks; rho=0.35; p<0.001 at PMA 32 weeks). We confirmed statistically significant differences in the FT4 serum level (p<0,001) between the 24-28 29-32 weeks GA groups. FT4 levels at PNA 2 weeks were 11.59 and 13.29 pmol/l for the 24-28 and 29-32 weeks GA groups, respectively. Despite this, the FT4 serum levels in the 24-28 weeks GA group compared to those at PNA 2 weeks and PMA 32 weeks were not statistically significant, while FT4 showed a gradual upward trend. We observed higher serum FT4 levels at PMA 32 weeks than at PNA 2 weeks. **Tables 4**, **5** show the 5^{th}-95^{th} percentiles for FT4 and TSH at PNA 2 weeks. **Figures 2**, **3** show the distribution of FT4 and TSH according to the GA.

Our findings reflect changes in the postnatal elevation of the serum FT4 level according to GA (**Figure 4**); these findings were similar to those reported by Williams et al.—the latter showed that FT4 elevation was attenuated in the 28-30 weeks GA group and was further attenuated in the 23-27 weeks GA group (15, 17, 18) compared with more mature preterm infants. The slow and gradual upward trend in our results of FT4 in the 24-28 weeks GA group might be related to the slow development and maturation of the hypothalamic-pituitary-thyroid axis (19). However, our results differed from Williams' reported data (18); the values of FT4 at PNA 2 weeks obtained in this study were lower than those in the above-mentioned studies for each GA, but were close to the data reported by Kilchemmann (17). The results obtained by Kilchemmann et al. revealed that the 50^{th} percentiles of FT4 were 0.95 ng/dl [11.19 pmol/l], 1.02 ng/dl [12.75 pmol/l], and 1.11ng/dl [13.86 pmol/l] in the 23-27, 28-30, and 31-34 weeks GA groups, respectively (17). Similarly, an increase in the level of thyroid hormones with increasing GA and PMA was also confirmed by Oh et al. (2). Based on the T3 serum level analysis and the conversion of FT4 to T3, the authors suggested that this increase might be related to the physiological maturation of the thyroid gland or the hypothalamic-pituitary-thyroid axis (2).

The second finding of our study was the determination of serum TSH levels; we observed no statistically significant between-group differences and no correlation with GA. This was similar to the results obtained by Imamoglu et al. (2015), who also did not observe any correlation between TSH levels and GA (20). As a final step in the comprehensive analysis of thyroid function, we estimated the ultrasound thyroid volume. Thyroid ultrasound is one of the most useful, non-invasive imaging tools that may determine the presence or absence of the thyroid tissue in its normal position, show the gland's overall morphology, and the presence of hemiagenesis or athyreosis. It is important to objectively assess the gland as normal, small, or enlarged by referring to the nomograms of the study population. Our study showed that the thyroid volume was positively correlated with weight in the 29-32 weeks GA group (rho=0.55; p<0.001) and in the whole study group (rho=0.25; p<0.001). Additionally, thyroid volume was positively correlated with FT4 at PNA 2 weeks (rho=0.25; p=0.019). These results are consistent with Ares's published data, where FT4 values were linearly correlated with the postnatal and postmenstrual age, and FT4 levels were also correlated with the thyroid gland volume (p<0.05) (21).

TABLE 5 | Distribution of TSH (mU/l) values in preterm infants born <33 weeks GA, measured at 2 weeks PNA.

	5p	10p	25p	50p	75	90	95
24-26 (n=19)	0.7795	1.222	2.0865	2.727	4.241	6.7402	7.8735
27-28 (n=20)	0.51275	0.5998	1.405	2.3135	4.29625	6.7303	8.7682
29-30 (n=33)	0.7994	0.8784	1.33	2.374	2.93	4.2384	6.278
31-32 (n=16)	0.68625	0.829	2.35	3.048	5.8415	10.9275	13.7055

In contrast, TSH serum levels were negatively correlated with thyroid volume (rho=-0.34; p=0.018). In this study, we determined the thyroid volume at PMA 32 weeks. The median thyroid volume for the 24-28 and 29-32 weeks GA groups were 0.26 (0.22, 0.31) and 0.27 (0.24, 0.34) ml, respectively. In their study of 57 preterm infants with a mean GA of 28.9 weeks and BW of 1181 g, Khan et al. showed serial ultrasound mean thyroid volumes ranging from 0.185-0.237 ml obtained between 13 and 64 days of life (22). These results are consistent with those of our study. In contrast, the results presented in our study were lower than those reported by Kurtoglu et al. The latter were 0.4 and 0.5 ml for preterm infants from the 25-28 and 29-32 weeks GA groups, respectively (12). Further studies are necessary to create nomograms for thyroid volume related to the GA of preterm infants.

During data collection, four subjects were diagnosed with thyroid disorders, and each received L-thyroxine treatment. Ultrasound imaging revealed hemiagenesis of the left lobe in two participants, which was predictive of thyroid disorders. We detected any abnormalities in the other two infants. Follow-up serum testing revealed that in one patient, levothyroxine treatment was discontinued after 1 year. In the whole study group, ultrasound of the thyroid gland revealed the presence of colloid follicles in the form of small 1–3-mm cysts. There were no other related abnormalities.

We would like to point out that an ultrasound examination of the thyroid gland is not required for diagnosis and possible treatment decision but in "doubt cases" it may constitute an additional reasonable factor contributing to a pragmatic conclusion. Given new technical possibilities, the use of good quality ultrasound probes of different sizes may improve diagnostic accuracy. The ultrasound evaluation may help neonatologists to take the decision about instituting a substitutive treatment. It is particularly important in the evaluation of infants referred with TSH elevation.

We have to highlight that a normal thyroid gland does not exclude the necessity of treatment.

The above examples point to the usefulness of the ultrasound examination.

Based on our results, we cannot confirm the impact of morbidities such as sepsis, NEC, ROP, or mechanical ventilation on thyroid hormone levels or thyroid volume. Similarly, Hemmati et al. did not find any significant changes in TSH and FT4 levels in critically ill neonates; Hemmati et al. showed transient elevation of TSH during recovery from the illness, but the FT3 and FT4 levels were not affected (23). In addition, our results showed no significant difference in FT4 and TSH values in preterm infants treated with dopamine or postnatal steroid administration in the out-of-schedule blood test time. Studies that investigated the use of drugs affecting

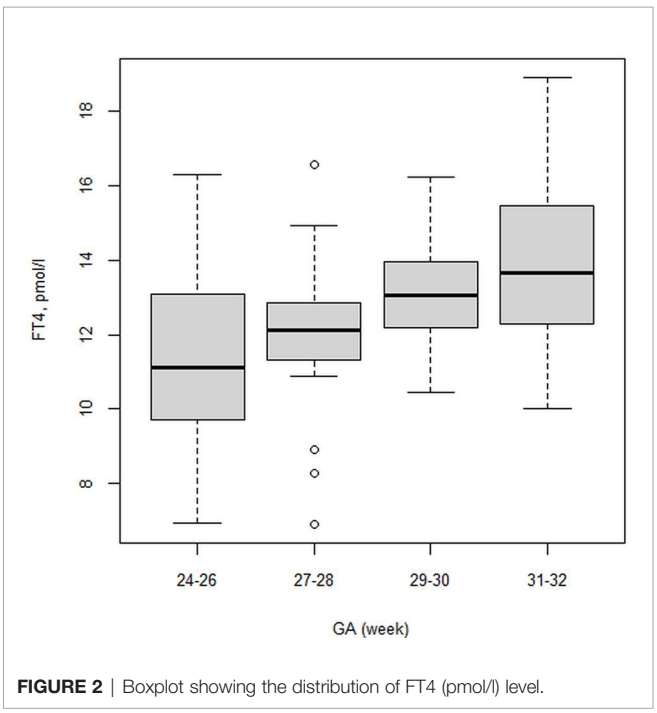

FIGURE 2 | Boxplot showing the distribution of FT4 (pmol/l) level.

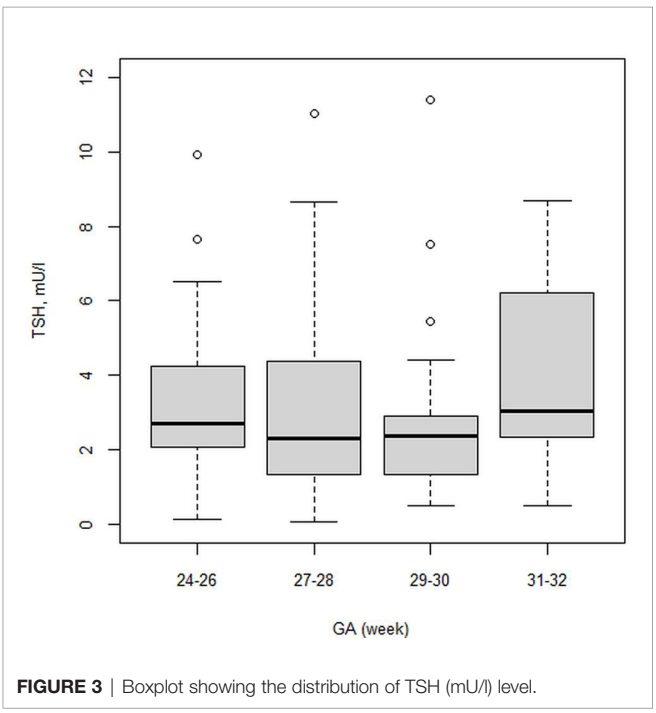

FIGURE 3 | Boxplot showing the distribution of TSH (mU/l) level.

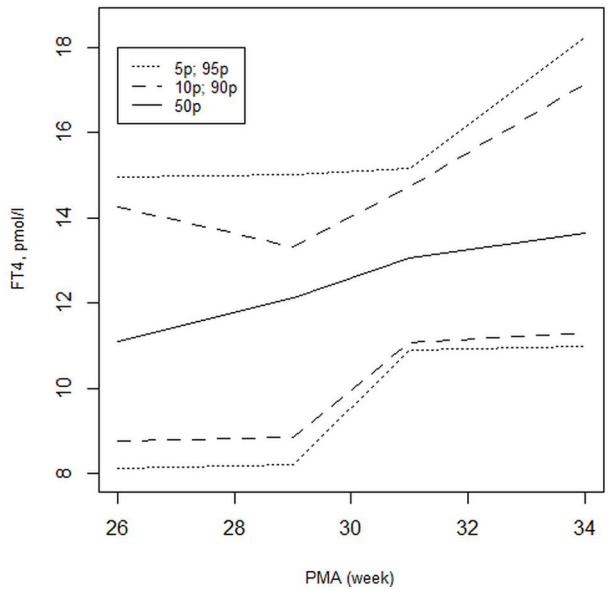

FIGURE 4 | Distribution of FT4 (pmol/l) values presented as the 5th to 95th percentiles for FT4 in preterm infants born with GA <33 weeks at PNA 2 weeks.

thyroid hormone levels, such as glucocorticoids and dopaminergic agents, confirmed that these drugs may diminish TSH secretion and FT4 serum levels (6, 7). Nonetheless, after discontinuation of dopamine, a quick reversal was observed in this study. Dobutamine does not influence the thyroid hormone profile (8). Similarly, after steroid discontinuation, an increase in the FT4 level was observed by Arai et al., but due to the small size of the study sample, some conclusions can be drawn from the data (7).

Nevertheless, due to improvements in the treatment and management of infants in the intensive care unit, the frequency of the incidence of THOP and delayed TSH in extra-preterm infants has declined. Limited data highlighted a significant difference in the thyroid status between preterm infants born with GA<28 weeks and those born more mature. In particular, controversies arise concerning the choice of treatment in preterm infants with BWs <1000 g or GA <28 weeks with incomplete development of the hypothalamus-pituitary-thyroid axis, as well as hypothalamic immaturity, therapeutic (24). It is not sufficient to distinguish THOP from central hypothyroidism, and the efficacy treatment with levothyroxine for persistent or profound TSH elevation or low FT4 levels in preterm infants remains unclear. Despite incomplete evidence, the current evidence seems to indicate that hypothyroxinemia in low birth weight infants should not be treated with levothyroxine as it fails to improve neurological outcomes (25, 26).

Most guidelines recommend levothyroxine treatment when the TSH serum level is >10 mU/l or the FT4 serum level is <10 pmol/l [0.8 ng/dl] at PNA 2-4 weeks (27, 28). A screening initiation of treatment should be implemented after persistent abnormal results measurements are taken 1-2 weeks apart (25, 29). Notably, preterm infants with BW <1000 g or born <28 weeks GA should undergo examinations on a case-by-case basis before levothyroxine treatment (27).

However, according to current knowledge, preterm infants with birth weight <1500 g or GA <32 weeks should be re-evaluated (30). Most researchers point out that after a primary screening test, rescreening of the measurement of the FT4 and TSH serum levels should be performed at the age of 2 and 4 weeks when a delayed TSH elevation is prominent. Some researchers recommend that a blood thyroid hormone test be performed at discharge (27).

This study is not without limitations. First, it was a single-center study with a relatively small sample size. Second, we did not evaluate the participants' iodine status, but pregnant women in Poland received an iodine supplement according to the Polish Endocrine Society's recommendation. Third, we did not obtain the expected results regarding the correlation between morbidity and FT4 and TSH values, which might have been related to the small sample size.

The study's main strength was the identification of the GA-specific distribution of FT4 and TSH atPNA p2 weeks and PMA32 weeks, as well as the identification of the ultrasound thyroid volume value for preterm infants.

In summary, ultrasound thyroid imaging might provide valuable insight into evaluating questionable thyroid disorders. This study's value cannot be denied as it allows the comparison of the thyroid gland's ultrasound size with thyroid function as expressed by FT4 and TSH serum levels.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Bioethical Committee of the Medical University of Warsaw (KB44/2019). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

AM and RB conceptualized this study. AM wrote the first draft of this manuscript. RB critically reviewed the manuscript and accepted the final manuscript for submission. KK: She was recruiting patients. All authors read and approved the final version of the manuscript.

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REVIEW
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An Overview on Different L-Thyroxine (l-T_4) Formulations and Factors Potentially Influencing the Treatment of Congenital Hypothyroidism During the First 3 Years of Life

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Congenital hypothyroidism (CH) is a relatively frequent congenital endocrine disorder, caused by defective production of thyroid hormones (THs) at birth. Because THs are essential for the development of normal neuronal networks, CH is also a common preventable cause of irreversible intellectual disability (ID) in children. Prolonged hypothyroidism, particularly during the THs-dependent processes of brain development in the first years of life, due to delays in diagnosis, inadequate timing and dosing of levothyroxine (l-thyroxine or l-T_4), the non-compliance of families, incorrect follow-up and the interference of foods, drugs and medications affecting the absorption of l-T_4, may be responsible for more severe ID. In this review we evaluate the main factors influencing levels of THs and the absorption of l-T_4 in order to provide a practical guide, based on the existing literature, to allow optimal follow-up for these patients.

Keywords: congenital hypothyroidism, L-thyroxine (L-T4), central nervous system, children, plastic period, pharmacological interferences

1 INTRODUCTION

Congenital hypothyroidism (CH) is a relatively frequent congenital endocrine disorder, due to the defective production of thyroid hormones (THs) at birth (1, 2). CH most commonly occurs because of disruption of thyroid gland development or a TH biosynthesis disorder (primary hypothyroidism); it can also be related to a defect in the release of thyroid-stimulating hormone (TSH), a condition which rarely occurs in isolation and is commonly associated with the deficiency of other pituitary hormones (combined pituitary hormone deficiency or CPHD) (secondary or central congenital hypothyroidism or cCH) (1, 2).

CH is also one of the most common preventable causes of irreversible intellectual disability (ID) in children (3) but early diagnosis and treatment can prevent serious consequences (4). THs are involved in the development of normal neuronal networks, and if CH is not treated it can lead to severe, permanent alterations in brain anatomy and function (5). It is important to consider that many complications, such as brain disorders and injuries and nerve growth retardation, may not be

clinically recognizable in the first weeks of life leading to delay in the introduction of treatments that can prevent disabling and severe outcomes (4, 6, 7).

Moreover, during pregnancy, absent or inadequate maternal levothyroxine (l-thyroxine or l-T_4) treatment correlates to an increased risk of obstetric complications at birth, as well as neuropsychological problems in the unborn child (8), adding to damage associated with neonatal CH.

Screening programs for CH are in place in many countries, allowing for the prompt initiation of treatment during the first months of life, frequently before clinical signs and symptoms appear (1). Generally, the recommended period for collecting blood samples for thyroid screening is between the 3rd and 5th day of life (9). The detection of a suspected thyroid dysfunction should be confirmed with secondary tests, which should be carried out promptly, ideally within the second week of life. However, despite the clear benefits of screening programmes, 70% of infants worldwide are born in areas without routine neonatal screening (10).

In CH, the incidence rate is reported to be about one in 2,000 - 4,000 live births, with a female-to-male ratio of nearly 2:1, although there are significant differences between countries (1–3): for example, in the United States, the incidence is 1 in 3500 to 5000, in Europe 1 in 3000 and in Japan 1 in 5700 (1–3). These differences are due to a number of factors including ethnicity, environmental influences, conditions affecting birth and pregnancy, and the presence or absence of screening programs (1–3). Moreover, sexual dimorphism can affect prenatal thyroid migration (11). The number of low birth weight (LBW) infants, who have a higher risk of CH, has increased in recent years due to improvements in neonatal intensive care (12).

Approximately two thirds of CH cases are secondary to thyroid dysgenesis which can involve both Mendelian and non-Mendelian inheritance: in fact, although CH is typically sporadic, familial cases have been described (1–3). In patients with a genetic form of CH, germline mutations in genes involved in thyroid gland organogenesis (i.e. *PAX8*, *TTF-1/NKX2.1*, *TTF-2/FOXE1* and *TSHR*) and responsible for thyroid dysgenesis are rarely found (1–3). Some cases of CH may result from inborn errors in the synthesis of THs (1–3). The clinical expression of mutations in some genes involved in disorders of thyroid function, such as *DUOX or DUOX2/DUOXA2*, may vary widely between individuals and over time; some patients who present thyroid function impairment in the first weeks of life do not require treatment (13). Initial l-T_4 doses and follow-up should be adapted to the needs of each CH patient taking into consideration the formulation used and THs levels.

Practices governing CH diagnosis and treatment have undergone significant changes in recent years: treatment with higher starting doses of l-T_4 is now the treatment of choice which has led to improvements in both auxological (14–16) and neurological prognoses (17, 18).

In all cases, there is a significant increase in TSH and/or a significant reduction in FT_4. Treatment should be promptly initiated with careful follow-up to determine appropriate dosage (13). Many guidelines (13, 19–21) for treating CH suggest a higher initial l-T_4 dose (generally at least 10-15 μg/kg/day) than the 5-10 μg/kg/day previously recommended, in order to normalize thyroid function more quickly (optimally within the first 14 days of treatment) and achieve better development quotient (DQ) scores. It appears that bone maturation at birth may predict psychomotor development in the first 12 months of life (22).

Several studies (13, 23–26) on the neurological outcomes of CH patients confirm that a rapid normalization of thyroid function can reduce the incidence of neurological impairment (19).

However, one study noted, for the first time, that children treated with high doses of l-T_4 were more likely to present hyperactivity, aggression and delinquency (27). Other data have also revealed that high serum T_4 levels may be associated with reduced attention in children of school age (28) and that there may exist a relationship between permanent attention deficit hyperactivity disorder (ADHD) and overtreatment in early years of life (29). These data show that overtreatment of CH can be dangerous and stress the importance of accurate follow-up to monitor dosage of l-T_4.

2 TREATMENT AND MONITORING OF PRIMARY CH

2.1 Goals of Treatment for CH

Triiodithyronine (T_3) is the biologically active form of TH but it is important to remember that as T_3 cannot pass the brain barriers in significant quantities, most T_3 in the brain is converted from local deiodination of T_4, meaning that in CH patients, it is not necessary to replace T_3 to normalise neurologic development (1). Research involving 47 infants treated with different doses of l-T_4, found that serum T_3 normalized and stayed normal regardless of dosage, indicating that l-T_4 alone is an adequate treatment for CH (30).

It is important to start treatment promptly in all infants who test positive in CH screening, after samples for confirmatory tests are taken, the results of which will determine the optimal l-T_4 starting dose (31). When the results of venous blood tests are not available on the same day, treatment should be started without waiting for the results, particularly if the patient's condition is a cause for concern (30, 32, 33),

The treatment goals for CH are outlined by the American Academy of Pediatrics (AAP) (30) and by the European Society for Paediatric Endocrinology (ESPE) (20) and can be summarized in the following main points:

- keeping serum Free T_4 (FT_4) above the average age-adjusted normal level but within the upper limit, especially in the first 12 months of life.
- keeping serum TSH below 5 mU/L.

Some data suggests that infants with serum T_4 levels under 10 μg/dL during the first 12 months of life, associated with serum TSH levels greater than 15 mU/L, have a lower Intelligence Quotient (IQ) than those with serum T_4 levels above 10 μg/dL (28). Furthermore, other

data show that treatment with higher doses of l-T_4 may be connected with higher IQ at 7 and 8 years of life, particularly in verbal working memory and sentence comprehension (27). Frequent and significant alterations in serum T_4 and TSH levels during the first 12 months of life are associated with differences in mental development index scores and verbal IQ (27, 33).

Prompt diagnosis and initiation of therapy for CH is essential for ensuring positive outcomes, especially in high-risk infants (34). However, some patients with CH may not achieve normal TSH concentrations despite l-T_4 treatment even at higher doses. Poor adherence to treatment is the most frequent reason for poor TSH control. Therefore, in patients who do not respond to treatment it is important to investigate whether l-T_4 is being correctly administered and if necessary, review the method of administration. The possibility of impaired l-T_4 absorption related to gastrointestinal diseases or the interference of drugs or other substances (see below) should also be considered (35).

Good therapeutic management practices appear to be of particular importance to ensure optimal results in terms of neuromotor and mental development, due to the THs-dependent processes of brain development in the first years of life. Professionals should provide clear explanations and instructions to caregivers to improve adherence to treatment (9, 34, 35). It is critical that families comprehend that treatment is essential for the best patient outcomes.

2.2 Dosage and Timing

Infants with severe forms of CH are at a higher risk for ID; for this reason, the dosage and timing of l-T_4 treatment are fundamental for optimising neurocognitive outcomes in the future. Delays in normalizing serum T_4 of more than one week can lead to lower IQ (23). Some data suggest that scores in behavioural and cognitive tests were lower for patients whose T_4 did not normalize before two weeks than for patients who achieved normal levels in under two weeks (24). Therefore, as stated above, the treatment goal is to bring serum T_4 to above below 129 mmol/L (> 10 μg/dL) as quickly as possible. The data available in the literature show that there is an inverse relationship between the initial l-T_4 dose, and the time taken to reach target serum T_4 levels (24) meaning that the prompt administration of an appropriate starting dose of l-T_4 is particularly important.

The starting l-T_4 dose recommended both by AAP and the ESPE is from 10 to 15 μg/kg/day (19–21, 30). In infants born at term this translates to an average starting dose of 37.5 - 50 μg per day (20, 21). In the above cited research, infants receiving 50 μg per day (corresponding to 12 - 17 μg/kg/day), versus 37.5 μg (corresponding to 10 - 15 μg/kg/day) performed better in tests measuring behaviour, reading, spelling and maths. The patients on the higher dose achieved IQ scores 11 points higher than those on the lower dose (24). Therefore, in infants whose serum T_4 levels are lower than 5 μg/dL, higher l-T_4 doses are recommended.

The goal is to normalize T_4 and TSH within 2 and 4 weeks, respectively (19, 24). T_4 and FT_4 concentrations should be in the upper half of the reference range for age, with normalization of TSH (19).

These positive outcomes associated with administering higher doses of l-T_4 have been highlighted by studies in Europe, the United States and Canada (24, 36–38). In one study, 83 infants were placed into three different groups, each receiving different initial doses of l-T_4 from diagnosis at birth (the first group was treated with 6.0 - 8.0 μg/kg/day, the second with 8.1 - 10.0 μg/kg/day, and the third with 10.1 - 15.0 μg/kg/day) and followed up at four years of age for growth and intellectual development. The infants with severe CH who started on the highest dose achieved the highest intellectual scores (39). Another study involving 61 infants comparing early treatment with delayed treatment and low doses with high doses, found that the outcomes for patients with severe forms of CH were more favourable in those treated early (under 2 weeks) and with higher doses (>9.5 μg/kg/day). Such patients presented normal psychomotor development at 10-30 months of age (23), showing that the time it takes for TSH to normalize is inversely correlated to neurological outcome (19).

Another study which analysed data on the intellectual development of 45 CH children found that differences and variations in serum levels of T_4 and TSH during the first year of treatment predicted scores on mental development index (MDI) and verbal IQ tests performed at 2 years and 6 years of age (33). It is clearly imperative to monitor patients so that l-T_4 doses can be promptly adjusted and optimal T_4 and TSH levels maintained.

2.3 Recommended Follow up During the First Three Years of Life

CH treatment must be carefully monitored with appropriate adjustment of the l-T_4 dose, until serum TSH and FT_4 concentrations have normalized. Clinical evaluations must be made every few months during the first three years of life together with frequent measurements of serum T_4 or FT_4 and TSH, in order to avoid prolonged periods of under- or overtreatment (19, 24). Management should focus on maintaining a status of euthyroidism, especially in the first 3 years of life when brain development is most dependent on THs. However, in CPHD patients with associated adrenal insufficiency or in patients in whom this problem cannot be excluded, adrenal function should be investigated, and glucocorticoid therapy should precede l-T_4, replacement treatment to avoid inducing an adrenal crisis (19).

The AAP recommends monitoring thyroid function 2 and 4 weeks after initiating l-T_4 treatment and every 1 to 2 months during the first 6 months of age. After this, patients should be monitored every 3-4 months between 6 months and three years of age, and then every 6-12 months until the child reaches adult height. Moreover, TH should be evaluated four weeks after any change in dose and follow-up evaluations need to be more frequent if results are abnormal or non-adherence to therapy is suspected (19).

Although in most infants, serum T_4 will normalize within seven to fourteen days and serum TSH will normalize within one month of treatment, some patients continue to present altered TSH levels (10-20 mU/L) or serum T_4 levels beyond these timeframes (40). This is often caused by under treatment,

although it is important to remember that in 10% of CH infants undergoing treatment the FT_4 feedback control on TSH secretion does not mature normally (41) due to the resetting of the pituitary-thyroid feedback mechanism *in utero* (42). In one paper reporting data for 42 CH paediatric patients, a prevalence of pituitary TH resistance of 43% in patients below one year of age was found. However, in childhood and adolescence this problem was found in only 10% of patients (42), suggesting that TH resistance is more common in younger patients and may decrease with age.

2.4 Effects of Inadequate or Non-Compliance

The availability of adequate TH is essential for normal brain development in the first two to three years of life. Low TH levels during this period can lead to irreversible damage, whereas after 3 years of age the effects of hypothyroidism can usually be reversed when the condition is treated. A study by the New England Congenital Hypothyroidism Collaborative highlighted the importance of regular monitoring and dose adjustments to control serum FT_4 and T_4 levels. Researchers found that eighteen infants included in the study with reduced serum T_4 levels, who were taking insufficient doses of l-T_4 (< 5 μg/kg/day) and who had an anamnesis showing poor compliance in the first three years of life, had a mean IQ of 87 (43). In comparison a larger group of correctly treated patients, with a history of normal serum T_4 levels, achieved a mean IQ score of 105.

Further data suggest that noncompliance after the first three years of life may also negatively affect cognitive achievement. For example, researchers made unannounced home visits on 14-year-old adolescents with CH and found that 44% were not adequately treated, having serum TSH > 15 mU/L and T_4 levels < 6.6 μg/dL in the majority (44). Psychometric tests revealed a mean IQ of 106. The importance of complying with TH treatment was explained to patients and their families (the dose was not altered), and 12 to 24 months later, serum TH levels had improved, and the repetition of psychometric tests showed an improvement in mean IQ of 6 points. Although the study did not include a control group, the authors concluded that non-compliance during adolescence is frequent, and that improved compliance can lead to improved cognitive function.

ID caused by CH is preventable but lack of awareness amongst caregivers about the benefits of treatment may be a barrier to clinical improvement in some patients (7). Newborn screening represents a unique opportunity for avoiding damage connected to CH (7).

2.5 Effect of l-T_4 Overtreatment

The effects of overtreatment with l-T_4 are well known (45–47). A prospective study by Rovet et al. (27) demonstrated for the first time that, despite an improvement in cognitive profiles, an initial high dose of l-T_4 can be associated with behavioural problems in childhood. Bongers-Schokking et al. (48) evaluated DQ scores at 11 years, showing that overtreatment can lead to a cognitive impairment of −17.8 points if it is prolonged and −13.4 points when the overtreatment is limited to the first 2 years of life. Another recent study by Bongers-Schokking et al. (29) concluded that episodes of overtreatment during the first 3 months can lead to permanent ADHD, while undertreatment may be related to autism.

3 TREATMENT AND MONITORING OF cCH

Many data and recently updated CH consensus guidelines support the treatment of cCH with a once daily administration of l-T_4 (13). In severe cCH (serum FT_4 levels before treatment below 5 pmol/L), the minimum l-T_4 starting dose should be no less than 10 μg/kg/day, in order to promptly bring FT_4 within the normal age-adjusted range (49). A lower starting dose of l-T_4 (from 5 to 10 μg/kg per day) should be used in milder forms to avoid overtreatment. For primary CH, the long-term biochemical aim must be to bring and maintain serum FT_4 within the upper half of the reference interval for the patient's age (49). Although it remains essential to conduct randomized clinical trials in children to obtain specific results for the paediatric population, data from adult studies support this approach (50, 51).

cCH should not be monitored by measuring serum TSH. Instead, serum FT_4 is the most reliable indicator of efficacy of treatment. If TSH levels prior to treatment are low, it is not necessary to take subsequent TSH measurements (13, 49).

Clinical and biochemical follow-up for patients with cCH should be similar to that for patients with primary CH. After diagnosis and the start of l-T_4 treatment, a first evaluation should be carried out within 1-2 weeks. If testing indicates a good response, subsequent evaluations should be scheduled every two weeks until serum FT_4 normalizes. After this phase, patients should be evaluated every 1-3 months during the first year of life, every 2-4 months during the successive two years, and then every 3-6 months. When it is necessary to take blood samples to measure serum FT_4, parents or caregivers must be instructed not to administer the daily dose of l-T_4 beforehand. If this is not possible, blood should be taken at least four hours after the last l-T_4 dose (13). For cases in which under- or overtreatment is suspected, it may be useful to measure TSH, and free or total T_3. Patients with FT_4 near the lower limit of the age-adjusted range should be considered at risk for undertreatment, especially if TSH >1.0 mIU/L while those with FT_4 near or above the upper limit of the age-adjusted range should be considered at risk for overtreatment, especially if there are typical clinical signs of thyrotoxicosis or high FT_3 levels (52).

Naafs et al. found that 86% of 92 Dutch children with cCH detected by newborn screening had both CPHD and ACTH deficiency and that 96% and 74% had GH and gonadotropins deficiency respectively (53). Most of the cases of ACTH deficiency (71%) were diagnosed in the first month of life, but GH deficiency on average was not diagnosed until 1.3 years of age. These findings demonstrate the importance of monitoring all children with cCH over time for the possible emergence of other pituitary defects. Naafs JC et al. (54) also reviewed studies on children with cCH

present in literature and found that the full-scale intelligence quotient was below 85 in 27% of patients, and below 70 in 10%. However, the age treatment was begun was not known for most patients (54).

4 PHARMACOKINETICS AND DIFFERENT FORMULATIONS OF L-T_4

The pharmaceutical preparation of drugs and the mode of administration are important elements in achieving and maintaining euthyroidism in CH and counteracting the effects of external influences on THs values.

Synthetic compounds of thyroxine usually contain the *laevo* isomer of thyroxine (l-T_4), most commonly as a sodium salt (55); however, although l-T_4 is frequently prescribed worldwide, the correct daily dosage, mode of administration, and approach to resolving treatment failure remain matters of debate. In primary CH, in order to maintain euthyroidism, thyroxine must be efficiently absorbed and unbiased serum TSH values must be available, which cannot be measured in patients with cCH.

Physiologically, l-T_4 is absorbed in the small intestine and, to a lesser extent, in the stomach (56). In patients with short bowel syndrome, the absorption of l-T_4 can be compromised (57). Although the pharmacokinetic parameters of l-T_4 have not been assessed in children, in adults there are differences between euthyroid and hypothyroid subjects. Hypothyroid subjects achieve C_{max} with a delay of 2-3h, associated with a decreased volume of distribution, and a higher bioavailability (than the standard 60–80%) (57–59).

l-T_4 permeation through the intestinal epithelium occurs principally in the duodenum and the jejunum, largely within 3 h after ingestion; absorption is most rapid within the first 2 h (57). Approximately 70% of the l-T_4 contained in tablets is absorbed and l-T_4 ingestion is best in the morning, at least 1 h before breakfast as fasting improves absorption (60).

Concomitant gastrointestinal diseases, such as coeliac disease (CD), *Helicobacter pylori* infection, lactose intolerance, inflammatory bowel diseases, as well as parasitic infestation (*Giardia lamblia*) may cause l-T_4 malabsorption (56, 57).

Increased gastric pH is also known to influence l-T_4 absorption (61); many studies confirm that individuals with impaired gastric acid secretion, as a result of disease or the use of proton pump inhibitors (PPI), often require higher l-T_4 doses to reach target TSH levels (62). In comparison, the available data suggest that pantoprazole and esomeprazole do not alter the pharmacokinetic parameters of l-T_4 absorption, although the evidence is insufficient for this to be certain (63).

Generally, the half-life of l-T_4 in a state of euthyroidism is 6-7 days (3-4 days with hyperthyroidism and 9-10 days with hypothyroidism). The full therapeutic effect of thyroxine is evident 3-4 weeks after treatment is initiated; if treatment is interrupted the therapeutic effect continues to be observed for 1-3 weeks. Because thyroxine has a long half-life, dose changes should not be made any more frequently than every 3-4 weeks (55).

Some studies also suggest that changing the drug's formulation can significantly reduce problems of l-T_4 malabsorption. Liquid l-T_4 is absorbed faster than solid pills (64), and has also been found to be less affected by gastric pH and conditions which impair l-T_4 absorption (61, 65). Liquid l-T_4 seems more effective in reducing TSH levels than tablets of the same dose, independently of whether malabsorption problems are present (65, 66). In patients assuming PPI or other interfering drugs, switching from tablets to a liquid formulation may improve the efficacy of treatment (67, 68). l-T_4 in soft gel capsules also dissolves better in increased pH (69) than tablets and appears able to reduce TSH levels both in patients with gastro-intestinal diseases and in patients without apparent malabsorption problems (70, 71).

Serum TSH is not always a reliable marker for pharmacological euthyroidism since values are affected by several drugs and some pathophysiological conditions (72, 73). However, it remains the best available marker as it is not easy to directly measure thyroxine absorption. The daily dose of l-T_4 required to obtain the desired TSH value/therapeutic effect does not directly correlate to the ingested dose as absorption of oral l-T_4 is between 60–80 %. Often high l-T_4 doses are necessary to attain target serum TSH concentrations, which can lead to costly hormonal monitoring. Repeated failure in reaching therapeutic targets is common. Dosage often has to be modified in children as they grow and gain weight, especially in the first year of life, although the need for higher does may also be due to impaired l-T_4 absorption.

It is important to consider the time of day that the drug is taken. Tablets should be taken at least 1 h before breakfast (60). In cases of reduced absorption or in patients with CD, lactose intolerance or other chronic inflammatory conditions, liquid l-T_4, or soft gel capsules may be better absorbed.

4.1 Different l-T_4 Formulations

l-T_4 is available in several forms (tablets, soft gel capsules, liquid and, in some countries, powder for preparing an intravenous solution). Unfortunately, tablets are the only form available in many countries.

4.1.1 Tablets

l-T_4 tablets are the oldest treatment for CH. l-T_4 is absorbed within 20–30 min after ingestion and the absorption process takes about 3 h (59). Taking tablets with food affects the drug's pharmacokinetics and the efficacy of treatment; t_{max} is delayed, and the peak value of l-T_4 absorption decreases (57, 74), as does drug bioavailability, from 15% to as much as 40% (75, 76). Not refraining from eating for at least 30 min after l-T_4 tablet ingestion, may result in significantly higher TSH levels (77). This is important to bear in mind in patients for whom persistent or frequent TSH alterations can be dangerous such as congenital hypothyroid patients, pregnant women, patients with cardiac disease, or oncological patients (78).

For young infants, the tablet should be crushed and mixed with breast milk or water. It should not be mixed with soy formula which interferes with absorption. If the infant requires

soy formula, l-T_4 should be given midway between feeds and thyroid function must be carefully monitored (79).

Although stable in dry air, l-T_4 becomes very unstable in the presence of light, heat and humidity. It is important to make sure that parents and other caregivers store the drug in its original container, away from sunlight and in a cool, dry place (55). Incorrect storage can affect bioavailability, making absorption more difficult which can cause fluctuations in the levels of THs.

4.1.2 Liquid Form

A liquid solution of l-T_4 containing ethanol is available in some countries. This form contains l-T_4, ethanol, and glycerine and appears to be more stable than tablets. Its main advantage is that the gastric phase of digestion is not necessary (64), meaning that it is absorbed more quickly (64) as has been confirmed by *in vitro* (80) and *in vivo* (81–84) studies conducted in hypothyroid adults assuming l-T_4 while fasting or with breakfast (81, 84). The faster onset of absorption, compared to tablets, minimizes the risk of drug-food interactions (64).

Studies conducted on paediatric patients affected by CH showed that the liquid formulation is a safe alternative to tablets, ensuring normal growth and neuromotor development (85–87). However, there appears to be greater suppression of serum TSH during the first months of treatment, indicating a higher risk of overtreatment (85–87).

Another concern about the liquid formulation is the potential risk of long-term side effects from the chronic administration of excipient ethanol; however, one millilitre of liquid formulation, equal to 100 μg of l-T_4, contains 243 mg of ethanol as excipient, more than four times lower than the safe threshold for alcohol established by the AAP (88). Several studies on treatment during pregnancy or lactation do not demonstrate any side effect on infants (89–91).

A recent multicentric Italian study involving 253 CH patients, with a follow-up period of up to 3 years, confirmed the efficacy of l-T_4 at high doses (87) in both liquid and tablet form. The data confirmed findings of previous studies by Cassio et al. and Peroni et al. (85, 86) and are in line with the results of a meta-analysis conducted by Aleksander et al., which showed that a high initial l-T_4 dose of between 10 and 15 μg/kg/day was effective and safe in all CH patients and that high FT_4 values during infancy did not correlate with IQ impairment at adolescence (18).

In a study by Vigone et al. patients treated with oral l-T_4 solution presented, after 15 days and 1 month, but not at 3 months, significantly lower median TSH and higher FT_4 values than those treated with tablets, with suppressed TSH levels in about 60% of the CH patients, indicating a higher risk of overtreatment during the first month of therapy (87). This may be due to a higher absorption of liquid l-T_4 suggesting that to avoid overtreatment, lower starting doses should be administered with shorter intervals between follow up visits (87). Both tablet and liquid l-T_4 were associated with adequate total DQ scores at 1 and 3 years of follow-up (85–87).

Another, prospective randomized control study in children with CH, to assess the efficacy and safety of liquid l-T_4 in comparison to tablets, evaluated thirty-nine children, aged 3-12 years old. At six months the group treated with liquid l-T_4 had higher TSH levels than the group treated with tablets, while FT_4 levels had no statistical difference. Dose adjustments were more frequent in the group treated with liquid. l-T_4 (92).

Finally, recently, another liquid formulation containing l-T_4, but without ethanol, using a calibrated oral syringe, has been studied, even if only in extremely premature infants (born below 28 weeks' gestation), without apparent effect on brain size (93).

4.1.3 Soft Gel Capsule

In this form, l-T_4 is dissolved in water and glycerine, and conserved in a gelatine matrix, to protect the hormone from degradation. It is free from lactose, gluten, alcohol, sugar, and dyes (94). The literature does not report studies in children with CH. One study, conducted in 60 euthyroid adults, investigated the effect of meals on the absorption of l-T_4 in this form. Patients who had been taking oral liquid l-T_4 with breakfast, switched to the soft gel capsule, without changing the mean dose. TSH, FT_4, and FT_3 levels were measured at the beginning of treatment with the soft gel capsule and after 6 months (95). There were no significant differences in TSH, whereas FT_4 and FT_3 levels were significantly lower in the subjects treated with the soft gel capsule (95). Another study found that, unlike l-T_4 tablets, the absorption of soft gel capsules is not affected by the concurrent consumption of coffee (96), suggesting that capsules may be of benefit for patients with CD, lactose intolerance and other chronic inflammatory conditions. A paper, evaluating these new formulations of oral l-T_4 for adults with central hypothyroidism, reported that liquid or soft gel l-T_4 was more effective at bringing serum FT_4 levels into the upper-normal range (97).

5 EFFECTS OF FOODS, DRUGS/ DISEASES, SUPPLEMENTATION, AND NUTRITION ROUTE ON THS LEVELS

Many physiological, nutritional, pharmacological or pathological factors can impair the intestinal absorption of oral l-T_4 (57, 98, 99). Patients, parents and carers often underestimate the risks of eating concomitantly or too soon after taking l-T_4 which can negatively affect absorption and therefore the efficacy of the therapy (100).

This is particularly worrying for paediatric CH patients, especially in the first three years of life given the thyroid-dependence of the central nervous system during this phase. Parents need to be made aware of the importance of correctly administrating l-T_4 to avoid interference from food and should be reminded to inform their child's paediatrician and endocrinologist about any drugs or medicaments the patient has taken or is taking.

Many drugs or medications can alter TH levels by affecting the hypothalamic and pituitary regulation of thyroid hormone production, the synthesis and secretion from the thyroid gland directly, the metabolism of THs (deiodination, sulfation and glucuronidation) or by altering affinity for levels of thyroxine binding globulin (100).

A number of drugs, such as proton-pump inhibitors, sucralfate and aluminium-containing antacids, affect the absorption of l-T_4 by changing gastric pH and reducing the solubility of tablets. Others, such as iron and calcium salts, phosphate binders, bile acid sequestrants and other resins, bind with l-T_4 to form insoluble complexes. The mechanism of interference for some drugs, such as raloxifene, is unknown (99). Certain herbal remedies also impair the ability to absorb l-T_4 (101) and nutritional supplements can also interfere, including infant formulas containing soy protein and iron, calcium and fibre supplements (100).

It is important to inform parents and caregivers about the possible interference of drugs and supplements and the potential damage they can cause in patients who are dependent on exogenous l-T_4 (100). The need to increase the dose of l-T_4 does not always signal gastrointestinal malabsorption but may indicate interference from food or medications (98). For this reason it is essential to take a patient's diet and medication use into consideration.

In a study of 925 adult hypothyroid patients aim at identifying factors with an effect on l-T_4 therapy, McMillan et al. found that over 50% assumed dietary supplements with a potential interaction with l-T_4, such as calcium (over 47% of cases) and iron (almost 12% of cases), while 68% reported frequent intake of foods rich in fibre, iodine, or soy (102). A study by Michel et al. (103) also found that many patients had insufficient awareness of the importance of l-T_4 administration schedules.

The problem is also of concern in paediatric patients, as dietary supplements are often given to infants and children (104). Some data suggests that more than 20-30% of children take dietary supplements regularly, most of which have not been recommended by a health care provider (104).

5.1 l-T_4 – Food Interaction

It is clear that interference from food can negatively impact the efficacy, and in some cases the safety, of treatment with l-T_4. This is also the case for liquid and soft gel capsule formulations (101). Most data, especially sporadic case reports, focus on adults and there is a need to augment our knowledge about the effects of food on l-T_4 in children. In the presence of problems of l-T_4 absorption, switching from tablets to liquid l-T_4 or soft gel capsules can in part solve the problem, and observing appropriate intervals between taking l-T_4 and eating can also reduce the possibility of interaction (105).

5.1.1 Infant Formula, Cow's Milk, and Breast Milk

In newborns and infants with CH, there is also the possibility of interference from infant formula. There are few data in the literature but infant formula milk contains fat, proteins and lactose that can cause l-T_4 to remain in the intestinal lumen which prevents it being absorbed (57). If patients are taking l-T_4 with foods, they may need larger doses to maintain euthyroidism (55). One study by Chon et al. demonstrated that in adults, cow's milk which is frequently ingested with breakfast can interfere with absorption (106). 1000 μg l-T_4 in tablet form alone or together with 355 mL of 2% cow's milk was administered to healthy adults. Peak serum TT_4 concentrations and the area under the curve were reduced by nearly 8% in patients taking l-T_4 with cow's milk (106). It is possible that breast milk, which contains only 20-30% of the calcium in cow's milk, is also decreases l-T_4 bioavailability (55). However, no data are reported in the literature for children.

5.1.2 Soy Based Formula

Some data, deriving mostly from old paediatric case reports, seems to show that soy infant formulas, as well as soy-containing baby foods, are related to altered thyroid function in early childhood (107–109).

Soy formula is often given to infants as an alternative to milk protein formula, especially in cases of intolerance to other formulas.

In 1959, Van Wyk et al. reported an infant fed with soy-based formula who presented cretinism and goitre at 10 months of age (108), which mostly resolved after the discontinuation of the soy-based diet. In another report on 78 patients under the age of one year, Pinchera et al. described the interaction between soya and l-T_4 in a 3-week-old child fed on soy-based baby formula because of suspected lactose intolerance. Despite high doses of l-T_4 (15 μg/kg per day), the infant's TSH values remained elevated (248 mIU/L). Only after substituting the soy-based formula with another formula, did TSH decrease (110).

Since the 1960s soy formulas have been supplemented with iodine and it wasn't until 1995 that other cases of soy-induced goitres in infants were reported (111, 112). Conrad et al. studied the influence of diet on thyroid function in paediatric CH patients, to investigate whether soy formula-based feeding can prolong the presence of increased TSH. The study included eight children on a soy formula diet and 70 on a non-soy diet. The children fed the soy formula had a more prolonged increase in TSH levels than children on non-soy formulas (113). The authors emphasized the need to frequently control levels of TSH in infants fed with soy formulas so that l-T_4 dosage can be adjusted accordingly.

In vitro studies indicate that phytoestrogens may affect the synthesis of T_3 and T_4 by inhibiting thyroid peroxidise (114). However, in a meta-analysis of 14 studies, Messina and Redmond found no significant adverse clinical effects on thyroid function in healthy adults but the l-T_4 dose might need to be increased in hypothyroid patients. For infants below the age of three years with CH (115), maximum attention appears necessary, given the thyroid-dependence of the central nervous system.

5.1.3 Fruit Juices

Some data suggest that fruit juices - particularly grapefruit, orange, and apple - also interfere with l-T_4 treatment by blocking the transporters carrying l-T_4 from the small intestine into the blood (116, 117). Lilja et al. (118) described an adult hypothyroid female, previously treated with l-T_4 with success, who presented a sudden new increase in TSH (more than 60 mU/L) with a reduced FT_4 concentration (6.4 pmol/L) after consuming grapefruit juice. Her hormone levels returned to within the normal range only after the woman reduced her consumption of this juice. These results confirm the data of a

randomized study of 10 healthy volunteers who drank grapefruit juice 1 h before taking l-T_4,and presented a reduction of 9% in l-T_4 absorption (118). Meyer et al. observed that THs upregulate expression of the transporter OATP2B1 hypothesising that hypothyroidism influences the interaction of juice and l -T_4 (119). Recently Tesic et al. reported a 31-year-old female patient with persistent hypothyroidism despite treatment with high doses of l-T_4 (alone or in combination with liothyronine) who had been taking l-T_4 tablets with juice or mint tea. The patient was advised to take l-T_4 with water, and her TSH levels normalized and her fT_4 levels increased in a few days (120). Although the data are scarce and, at times, conflicting, it seems prudent not to take l-T_4 with juice, but it does not seem necessary to eliminate fruit juice consumption completely.

Deiana et al. (121) reported a 37-year-old patient treated with l-T_4 and with euthyroidism after thyroidectomy, who presented an unexpected TSH increase after eating large quantities of papaya (5-6 fruit a day). She was advised to stop eating the fruit completely and after 45 days her serum TSH concentration returned to within the normal range. Papain reduces gastric acid secretion which lowers l-T_4 absorption; other components of the fruit, as well as fibre terpenoids, saponins, alkaloids, and flavonoids, may also reduce l-T_4 absorption (121).

5.2 l-T4 – Drugs/Diseases Interaction

Many conditions or drugs may influence the gastric environment and affect the absorption of l-T_4. Tablets of l-T_4 need to be dissolved in an acid environment. Subjects with achlorhydria, reduced gastric acidity or who are taking PPIs do not have the ideal gastric environment to dissolve l-T_4. PPIs are increasingly prescribed to children (122) and are effective treatments for many gastric diseases, duodenal ulcers, non-steroidal anti-inflammatory-induced ulcer-related prophylaxis, *Helicobacter pylori* in combination with other medications, gastro-esophageal reflux disease and eosinophilic esophagitis. They are also prescribed for functional dyspepsia, chronic cough, and infantile reflux (122).

In adult goitrous patients taking l-T_4, omeprazole assumption led to significant increases in serum TSH; this effect was reversed by increasing the dose of l-T_4 or by discontinuing the use of omeprazole (123). Similar results have been reported for lansoprazole, but not pantoprazole or esomeprazole taken for one week (57). Antiacids also appear to interfere with thyroxine absorption by influencing gastric pH (57).

The recently developed liquid l-T_4, dissolved in an alcoholic solution, does not need an acid environment as it can be directly absorbed through the intestinal mucosa (124).

Besides drugs, pre-existing malabsorption diseases or disorders that impair gastric acidity also affect the bioavailability of l-T_4 (125). There are some reports of elevated serum TSH levels in patients with coeliac disease and inflammatory bowel diseases taking doses of thyroxine that were previously sufficient to normalise serum levels (125).

Some studies report similar findings for patients with *Helicobacter pylori* infection and atrophic gastritis – both of also which impair gastric acidity (125).

Several reports regarding cases of l-T_4 malabsorption in patients with various manifestations of CD have been reported (125, 126). For example, in a study conducted on seventy-nine children with permanent CH, 6 patients (4 girls, 2 boys) were positive for CD antibodies, showing a higher-than-normal prevalence of this disease in children with permanent CH. The available data show that a gluten-free diet results in the reduction of l-T_4 dose requirements (127). Because the symptoms of some forms of CD are subtle, several authors suggest screening with CD markers in patients with hypothyroidism who need higher than normally expected doses of l-T_4.

Franzese et al. report an infant with CH who developed, during l-T_4 replacement therapy, a cow's milk protein intolerance and subsequently CD. Both milk protein intolerance and CD affect the intestinal absorption of l-T_4, making the management of CH difficult. After starting a diet with a hydrolyzed milk protein and maintaining the dose of l-T_4 at 12 μg/kg dose, serum T_4 improved and TSH decreased (128). A case of l-T_4 malabsorption with lactose intolerance was reported in a 55-year-old woman with primary hypothyroidism whose TSH levels were persistently high (128).

After being diagnosed with oligo-symptomatic lactose intolerance, the patient was given a lactose-free formulation of l-T_4 (150 mg daily) and began following a lactose-restricted diet; after 3 months the problem had resolved (129).

The presence of a pylori infection and atrophic gastritis of the body of the stomach, causing bacterial production of urease that neutralises gastric pH, may determine a decreased TSH suppression (123).

An infant with confirmed CH taking l-T_4 experienced a possible drug interaction with simeticone (130) which is used to treat infant colic, a condition frequently seen by paediatricians. In this case, despite adequate l-T_4 dosage, TSH was high, suggesting undertreatment. This case highlights the importance of regularly reviewing a patient's clinical history and considering the possibility of drug interactions in unusual circumstances, especially where patients need high doses despite good compliance and proper administration (131). Clinicians should alert the parents of CH children starting l-T_4 about the use of medicines containing simeticone (130).

Small intestinal bacterial overgrowth (SIBO) is a heterogeneous condition with nonspecific symptoms characterized by the presence or increase of atypical bacteria in the small intestine that unbalances intestinal microbiota. SIBO is characterized by symptoms such as flatulence, distension and abdominal pain. These symptoms might be indistinguishable from those presented by patients with functional gastrointestinal disorders. In most cases, SIBO is associated with motility or inflammatory disorders (132). The abnormally high levels of bacteria in the small intestine can cause malabsorption and suboptimal responses to narrow therapeutic index medication which are absorbed in the small intestine, such as l-T_4 (133). In one study on patients with SIBO, l-T_4 tablets and a compounded oral suspension were not absorbed efficiently, resulting in below optimal control of serum TSH. When patients switched to l-T_4 sodium oral solution, TSH levels fell and symptoms resolved (133).

Malabsorption of l-T_4 has also been described in patients infected with *Giardia lamblia*. Giardiasis is a common intestinal infection, but it has only rarely been reported as a cause of impaired l-T_4 absorption (124). Giardiasis is probably under-diagnosed; in developing countries with poor sanitation its prevalence is about 20% while in industrialised countries prevalence ranges from 3% to 7% (124). Intestinal giardiasis can cause maldigestion, malabsorption and diarrhoea, leading to anaemia, weight loss and growth retardation (124). In cases of giardiasis infection in patients taking l-T_4, switching from tablets to oral solutions revert decreased l-T_4 absorption (124).

Short bowel syndrome (SBS), a major cause of intestinal failure in children, is diagnosed when the length of the small intestine is 25% shorter than expected for the child's age (131). In paediatrics, the most frequent causes of SBS are resections secondary to necrotizing enterocolitis, gastroschisis, intestinal atresia and intestinal volvulus (134). SBS can cause a number of metabolic changes in the body, which can affect growth, intestinal adaptation and lead to metabolic bone disease as well as an impaired functioning of the hypothalamic-pituitary-thyroid (HPT) axis (135). Passos et al. reported six consecutive cases of children with SBS with associated hypothyroidism during the period of intestinal rehabilitation (135).

5.3 l-T4 – Dietary Fibre

A fibre-rich diet or dietary fibre supplements may also significantly reduce the bioavailability of l-T_4 due to a non-specific link to fibre in the intestinal lumen causing malabsorption of the hormone (125). Products containing insoluble dietary fibre increase bowel motility, potentially altering the intestinal absorption of l-T_4 (78). Although not all authors agree (135), in cases of dietary modifications which increase fibre intake, it may be necessary to monitor TSH levels and increase the dose of l-T_4 (136).

5.4 l-T4 – Essential and Trace Elements

The possibility of interactions between levothyroxine and essential and trace elements has been investigated. Di- and trivalent elements, especially calcium and iron, appear to decrease l-T_4 bioavailability (137). The mechanisms involved are not entirely clear but may connected to unspecific adsorption and the formation of insoluble complexes in the intestine (138). Parents should thus be informed about the possible adverse effects of calcium and iron supplementation.

5.4.1 Calcium

CH may occur in association with congenital parathyroid hormone (PTH) insufficiency which leads to hypocalcaemia and low secretions of PTH (138). The combination of CH and PTH insufficiency occurs in patients with 22q11 microdeletion/DiGeorge syndrome, or due to PTH resistance in pseudohypoparathyroidism (139, 140). Metabolic bone disease is very frequent in preterm infants, occurring in 20-30% of very LBW infants (VLBW, <1500 g) and in 50-60% of extremely LBW infants (ELBW, <1000 g), who are at an increased risk for CH (141). The introduction of calcium-based supplements to improve PTH insufficiency could interfere with the absorption of l-T_4.

In adults, calcium carbonate may reduce l-T_4 absorption, resulting in a significative reduction of FT_4, higher TSH levels in 20% of patients. These TH alterations were normalized after calcium carbonate discontinuation (142). There are several instances in the literature of interaction between calcium carbonate and l-T_4 (143), and in a large observational study on patients assuming l-T_4 tablets or iron supplements, in which the authors observed a significant increase of serum TSH in 4.4% of patients in the first group and 7.5% in the second group, concluding that supplementation with calcium and iron may cause a reduction in the absorption of l-T_4 (62).

Other calcium preparations also potentially interact with l-T_4. Diskin et al. (144) looked at TSH levels in more than 60 patients assuming l-T_4 with different phosphate binders (mean dose 95–98 μg/day). The reported that only calcium carbonate, but not calcium acetate, resulted in significantly higher TSH levels. Zamfirescu et al. (145) studied the effect of calcium formulations (acetate, citrate, and carbonate) delivering a dose of 500 mg of elemental calcium on the absorption of l-T_4 tablets at a dose of 1000 μg in eight healthy adults. For all the calcium preparations, taking the calcium supplement at the same time as the l-T_4 tablets reduced absorption of l-T_4 by 20 to 25%. The researchers stressed the importance of taking the examined calcium formulations and l-T_4 at different times.

Morini et al. (146) studied a cohort of 50 postmenopausal women with hypothyroidism taking co- calcium supplements containing elemental calcium with a dose of 600-1000 mg for day. When the supplements were taken at the same time as or within 2 hours of l-T_4 ingestion, the majority of patients presented significant increases in TSH levels, blood pressure, total cholesterol levels, and fasting glycemia.

Some data suggest that in adults the interaction of l-T_4 and calcium can be reduced by replacing l-T_4 tablets with liquid l-T_4. Benvenga et al. reported data for 12 hypothyroid patients taking calcium carbonate (1000 mg/day) and assuming tablets or liquid l-T_4 formulations, concluding that liquid l-T_4 may be more resistant to sequestration by calcium (147). Mazokopakis et al. (148) found that only 8.4% of more than 150 patients were taking calcium carbonate at least 4 h before or after l-T_4.

5.4.2 Iron

Anaemia, defined as a haemoglobin levels of two standard deviations below the mean for age, is prevalent in infants and children worldwide with microcytic anaemia due to deficient iron intake being the most common type in children (149). Iron deficiency anaemia (IDA) can lead to cognitive problems that can be prevented or ameliorated with iron supplements or increased intake of iron in food (149). The phenolic, carboxylate, and amine functional groups on l-T_4 molecules enable them to react with ferrous salts to form insoluble or only partially soluble complexes, which reduces the absorption of l-T_4. Iron also plays an important part in TH synthesis and amino acid metabolism (150). Physiologically, IDA is able to impair THs metabolism, decrease T_4 and T_3 levels, reduce the peripheral conversion of T_4 to T_3 and T_3 metabolism and decrease hepatic T4-5'-deiodinase leading to an increase in circulating TSH activity (151).

Anaemia is often present in infants affected by CH with severity depending on the degree of neonatal hypothyroidism which, if present during development, can lead to persistent health problems also after thyroid replacement therapy is started (152). "Uncomplicated" anaemia secondary to hypothyroidism responds to thyroid replacement therapy alone (153). Anaemia in hypothyroidism must be thoroughly evaluated because treatment must address its cause.

Gökdeniz et al. (154) showed that more than 15% of children with IDA presented concomitantly subclinical hypothyroidism, and Metwalley et al. (155) observed that primary school children with IDA were likely to develop SH and intellectual dysfunction. The presence of anaemia could influence the outcome of l-T_4 therapy. Campbell et al. reported a lower efficacy of l-T_4 treatment in adults when the drug was administered together with ferrous sulphate, with an increase in TSH levels in 79% of patients (156). A reduced absorption of l-T_4 due to the co-administration of ferrous sulphate has been reported in a number of patients (157). Shakir et al. (158) suggest that interaction can occur even if patients maintain an interval of 4–6 h between taking l-T_4 and supplements or medicines containing iron. Leger et al. report a 60-year-old female, previously treated with success with l-T_4, who developed hypothyroidism after starting to take ferrous fumarate daily (TSH 243 mU/L, T_4 <0.52 pmol/L). The woman's thyroid function normalised 2 months after she stopped taking the iron supplement (159). Finally, Atruktsang et al. (160) recorded the time taken to achieve euthyroidism in over 600 subjects taking l-T_4 as well as the number of dose adjustments that were necessary; among the patients whose dose need to be adjusted three or more times, a significant number used ferrous supplements.

Some data seem to suggest that, as with calcium, the use of an oral liquid form of l-T_4 can help reduce problems of absorption correlated to iron supplementation. For example, in a small population of hypothyroid patients, Benvenga et al. (147) investigated l-T_4 interaction with iron, observing a significant decrease in TSH levels in those who switched from tablets to oral liquid l-T_4.

5.4.3 Aluminium

Many antiacids contain aluminium. A number of case studies in adults report l-T_4 malabsorption caused by the concomitant use of aluminium hydroxide (161). Liel et al. investigated five hypothyroid subjects treated with l-T_4 who took gel tablets containing aluminium hydroxide for 2-4 weeks, observing a significant increase in serum TSH during the period of aluminium hydroxide ingestion (138).

5.4.4 Iodine

Iodine is essential for THs which play an important role in foetal development and early infancy. Iodine deficiency in children may lead to reduced physical and intellectual capacity (162). In iodine-deficient areas, CH caused by iodine deficiency has a significant incidence (34). Preterm infants are at particular risk, also because parenteral nutrition and preterm infant formulas, including those used in hospital settings, do not always provide adequate iodine (163). Excess iodine intake may also cause a physiological decrease in THs synthesis, which is usually transient, due to the Wolff-Chaikoff effect (164). The thyroid's ability to recover from the Wolff-Chaikoff effect is not fully mature until 36 to 40 weeks of gestation, meaning that preterm infants have a greater risk of prolonged hypothyroidism. Sources of excess iodine include iodine-containing antiseptics, radiographical contrast agents, and excess maternal intake of iodine (from diet or supplements) transmitted to infants in breastmilk (34).

The main dietary sources of iodine are iodized salt, seafood, and dairy products (165). Seaweeds are rich in iodine but the iodine content of seaweeds and dairy products varies considerably (166). Sea salt, Himalayan salt, and salt used in food processing is not always rich in iodine (148). Foods such as soybeans, cruciferous vegetables, and sweet potatoes contain substances capable of interfering with the thyroid's uptake of iodine but in healthy individuals with adequate iodine intake, consumption of these foods does not lead to thyroid dysfunction (115). Vegans should use iodized salt and/or consume sea vegetables to minimise their risk of developing iodine deficiency (165).

The aetiology of hypothyroidism is multifactorial, but the most frequent causes are excess iodine intake and/or critical illness. Exposure to iodinated Contrast Media (iCM) is an increasingly common source of excess iodine in vulnerable patients (166). The thyroid gland has two auto-release mechanisms to manage high intra-thyroid iodine doses, namely the *Via* the Wolff–Chaikoff effect and the escape phenomenon (167). However, in newborns and infants these mechanisms are not mature making them prone to iCM toxicity (168). Infants with iCM toxicity typically have low FT_3 plasma concentrations with low or normal FT_4 and low or normal TSH, consistent with a non-thyroidal disease syndrome.

5.4.5 Selenium

The enzymes, iodothyronine deiodinases, glutathione peroxidases, and thioredoxin reductases, involved in TH biosynthesis and metabolism, regulation of the redox state, and protecting the thyroid from oxidative damage are selenoproteins. Thus, low selenium levels may lead to hypothyroidism: see below in the section related to parenteral nutrition (169).

5.5 l-T_4 – Vitamin Interaction

It has been shown that increased gastric pH can influence the absorption of l-T_4. Jubiz et al. studied the effects of vitamin C, capable of lowering gastric pH, on l-T_4 absorption (170) They conducted a study in 31 hypothyroid subjects with gastritis under l-T_4 therapy, taking a median dose of 100 μg in tablet form, with 120 mL of water with or without 500 mg vitamin C in solution. While on vitamin C, TSH levels fell in all patients, and FT_3 and FT_4 levels increased significantly. Antúnez et al. (171) observed similar results in 28 patients with elevated TSH levels, despite being on a l-T_4 dose higher than 1.70 μg/kg. Patients were asked to take l-T_4 tablets with 1 g of vitamin C (effervescent tablets, dissolved in 200 mL of water) for 6–8 weeks. Significant decreases in serum TSH levels (from 9.01 ± 5.51 mU/L to 2.27 ± 1.61 mU/L) were achieved.

Biotin is a vitamin commonly used in multivitamin preparations and used to treat progressive multiple sclerosis, several inherited metabolic diseases and acquired dermatologic diseases. It can interfere with many endocrine laboratory assays (including TSH and FT_4) (172), which can lead to erroneous or delayed diagnosis of CH. There are cases in the literature of children taking high doses of biotin as therapy for metabolic disease being wrongly diagnosed with hyperthyroidism (172).

The correct assessment of thyroid function may be influenced by biotin therapy (173). High levels of biotin–streptavidin can compete with biotinylated components leading to inaccurate and misleading results characterised by increased FT_3, FT_4, levels and a reduction in TSH levels, which may lead to misplaced suspicion of Graves' disease (173). This could lead to erroneous therapeutic strategies with serious consequences in the first three years of life given the thyroid dependency of the central nervous system in this period.

Wijeratne et al. conclude that interference peaks at approximately 2 h after biotin ingestion and can persist for up to 24 h (174). In a newborn with trisomy 21, CH and partial biotinidase deficiency, because of interference with the TSH assay from concurrent biotin administration routine screening detected "normal" TSH levels leading to a delayed diagnosis of CH (172).

5.6 l-T_4 – Nutrition Route

In paediatrics, the most basic and important method of nutritional intervention is enteral nutrition (EN) (175). EN is introduced for patients who cannot meet their energy and nutritional needs through normal feeding. EN is often required for children with growth retardation, inadequate weight gain, or weight deficit, and is employed in the treatment of diseases such as Crohn's, and food allergies or intolerance (175). In neonates, EN is required in situations such as premature or necrotizing enterocolitis (175).

Reis et al. (176) carried a multicentre study in Brazil to evaluate possible drug-EN interactions. The authors found l-T_4-EN interaction to be one of the most frequent and clinically significant interactions. In an earlier study, Dickerson et al. (177) evaluated 13 hypothyroid patients in hospital: all participants received EN; their l-T_4 doses were kept the same as they were before the patients were hospitalised for 20 ± 5 days. Eight of the thirteen patients developed subclinical or overt hypothyroidism, indicating that it is vital to monitor hypothyroid patients who receive continuous EN and who are being treated with l-T_4. Manessi et al. (178) discovered that l-T_4, may be adsorbed by enteral feeding tubes and hypothesised that this mechanism was related to a reduction in treatment efficacy. However, Wohlt et al. (179) concluded that the amount of l-T_4 absorbed by feeding tubes is likely to be clinically irrelevant, and that impaired l-T_4 absorption may be caused by the concomitant ingestion of food. Pirola et al. (180) studied 20 euthyroid patients, a day after surgical invention, to compare the efficacy of different formulations of l-T_4 administered while patients were attached to an enteral feeding tube. EN was stopped for 30 min before and after powdered l-T_4 tablets were administered, whereas liquid l-T_4 was administered *via* the feeding tube without interrupting EN. There were no significant differences in the results obtained by the two methods, leading the authors to conclude that EN does not impact the absorption of liquid l-T_4.

Parenteral Nutrition (PN) is used when normal and enteral feeding are impossible (181). Some reports show that long-term PN is a risk factor for low iodine and/or selenium levels, causing hypothyroidism, which in some cases is severe, stressing the importance of dosing of these elements and evaluating THs (182, 183).

6 EFFECTS ON THYROTROPIN RELEASING HORMONE (TRH) AND TSH LEVELS

Drugs and medications frequently influence thyroid function altering the secretion of TRH or TSH (184), even if only a small subgroup (glucocorticoids or GCs, dopamine agonists or DAs, etc) affects the hypothalamus or pituitary (100). **Tables 1** and **2** report the drugs and medications which affect thyroid function in children with intact an hypothalamus-pituitary-thyroid axis (**Table 1**) and in those dependent on exogenous l-T_4 (**Table 2**), also describing the mechanisms of action.

Generally, the widely used GCs are not able to induce clinical central hypothyroidism even when used for long periods at high doses. DAs do not typically lead to clinically evident central hypothyroidism, but in patients with nonthyroidal illness they may cause an additional TSH suppression, which could potentially result in iatrogenic central hypothyroidism. Rexinoids, in contrast, frequently induce clinically relevant central hypothyroidism in the majority of subjects, who will require l-T_4 treatment and follow up to control serum FT_4. This new type of drug is likely to be used on increasing numbers of patients (advanced cancer, metabolic disorders, dermatologic disorders), and it is therefore important to consider the risks of possible side-effects so that they can be managed with treatment.

l-T_4 is also the first line treatment for secondary hypothyroidism, which in developing countries is frequently caused by CH, Hashimoto thyroiditis, thyroidectomy, or iodine deficiency (185). The interference of food on l-T_4 absorption, as previously mentioned, is known to affect and the efficacy, and in some cases, safety of therapy but there is a lack of awareness among patients and health care workers about this potential problem.

6.1 Glucocorticoids

Glucocorticoids are often used in paediatrics for their immunosuppressant and anti-inflammatory properties, individually or in combination with other drugs both short and long term (186, 187). It is well known that GCs affect serum TSH levels in animals and humans, reducing TSH secretion by directing effecting TRH a hypothalamic level (188). Physiologically, it appears that levels of hydrocortisone have a key role in the diurnal variation of serum TSH, with lower levels in the morning and higher levels at night (189, 190). Some data have shown that high dose GCs may suppress serum TSH in normal subjects and also in hypothyroid patients (191).

TABLE 1 | Medical conditions, drugs and medications potentially affecting l-T_4 absorption in children.

Diseases	Drugs and medications
Coeliac disease	Acid suppression therapies (proton pump inhibitors, H2 receptor antagonists, sucralfate)
Lactose intolerance	Bile acid sequestrants (cholestyramine, colestipol, and colesevelam)
Atrophic gastritis	Calcium salts (carbonate, citrate, and acetate)
Helicobacter pylori	Ferrous sulphate
Chronic cholestasis and liver cirrhosis	Multivitamins (containing ferrous sulfate or calcium carbonate)
Short bowel disease	Cation exchange resins (Kayexelate)
Inflammatory bowel disease	Simethicone
Intestinal surgery	Ciprofloxacin
Giardiasis	β-blockers
Small intestinal bacterial overgrowth (SIBO)	Charcoal
Foods	
Ingestion with a meal (food intake)	Oral bisphosphonates
Cow's milk?	Phosphate binders (Sevelamer hydrochloride, aluminium hydroxide)
Dietary fibre	Rifampicin
Grapefruit	Polystyrene sulfonate
Soybeans and soy	Tricyclic antidepressant
Papaya	

TABLE 2 | Main drugs potentially affecting thyroid function or thyroid hormone levels in patients with congenital hypothyroidism.

Drugs	More frequent use in children	Effects
Iron	Anaemia	Inhibition of l-T_4 absorption
Calcium	Hypocalcaemia	Inhibition of l-T_4 absorption
Aluminum/magnesium hydroxide	Gastro-esophageal reflux (antiacid)	Inhibition of l-T_4 absorption
Sucralfate	Gastro-esophageal reflux (antiacid), gastritis, ulcers	Inhibition of l-T_4 absorption
Colestyramine	Chronic cholestasis/inherited hyperlipidemia	Inhibition of l-T_4 absorption
Colestipol	Chronic cholestasis/inherited hyperlipidemia	Inhibition of l-T_4 absorption
Carbemazepine/ Oxcarbemazepine	Seizures, mood disorders	TSH suppression; Increased hepatic metabolism
Glucocorticoids	Acute respiratory conditions as asthma and croups, inflammatory bowel diseases, nephrotic syndrome, etc	TSH suppression; inhibition of 5' deiodinase; decreased thyroxine binding globulin levels
Nonsteroidal anti-inflammatory medications	Fever, musculoskeletal pain	Displacement from thyroxine binding globulin (laboratory artifact)
Phenytoin	Seizures	Increased hepatic metabolism; displacement from thyroxine binding globulin (laboratory artifact)
Phenobarbital	Seizures	Increased hepatic metabolism
Lithium	Acute mania and bipolar disorder	Inhibition of T4/T3 secretion
Dopamine agonists	Hyperprolactinemia	TSH suppression
Propranolol	Cardiac dysrhythmias	Inhibition of 5' deiodinase
Rifampicin	Treatment of several types of bacterial infections	Increased hepatic metabolism
Furosemide	Renal, cardiac and peripheral oedema, ascites, hypertension)	Displacement from thyroxine binding globulin (laboratory artifact)
Iodide	Drugs containing iodide	Inhibition of 5' deiodinase
Amiodarone	Cardiac arrhythmias	Inhibition of 5' deiodinase, Thyroiditis
Nicotinic acid	Paediatric lipid disorders?	Decreased thyroxine binding globulin levels
Methadone	Life-Limiting Illness?	Increased thyroxine binding globulin levels

Nevertheless, long-term high doses of GCs or the cortisol excess typical of Cushing's syndrome do not seem to result in clinical central hypothyroidism necessitating l-T_4 treatment (192). Dexamethasone doses as low as 0.5 mg or prednisone 30 mg can reduce TSH levels significantly (192).

This suppressive effect of GCs on TSH secretion appears to be controlled by the hypothalamus through the inhibition of TRH (193). In humans, GCs receptors are present in the TRH neurons of the paraventricular nucleus and high dose GCs can reduce TRH mRNA levels in the hypothalamus, acting on the TRH gene, probably the primary mechanism for lowering TSH secretion from the hypophysis (194).

The effect of GCs on TSH should be taken into consideration when checking thyroid function in CH patients, in order not to mistake an overtreated condition with a physiological response to GC treatment.

6.2 Dopamine

Dopamine is widely used in critical illnesses to increase blood pressure, cardiac output, urine output, and peripheral perfusion

in neonates, infants, and older children with shock and cardiac failure (195).

Dopamine and dopamine agonists can suppress serum TSH and affect the activation of dopamine D2 receptors, reducing TSH pulse amplitude without significantly altering TSH pulse frequency (196, 197). Interestingly, in rats, dopamine stimulates hypothalamic TRH release, but because its overall effect is to lower serum TSH, the inhibitory effect on the pituitary exceeds the first effect (198).

Data on neonates with nonthyroidal illness (NTI) syndrome treated with dopamine infusions indicate that dopamine and NTI have an additive effect on suppressing the HPT axis, placing these individuals at risk of iatrogenic central hypothyroidism (199, 200). It is not clear whether treatment with l-T_4 should be given to patients with NTI who are receiving dopamine infusions but, given the thyroid dependence of the central nervous system, the risks of not treating children with CH under 3 years of age should be carefully considered.

6.3 Antiepileptic Drugs

Several drugs used to treat epilepsy such as carbemazepine (CBZ), oxcarbemazepine and valproic acid (VPA) could increase the metabolism of TH through the hepatic P450 system and could also impede pituitary responsiveness to hormonal feedback and cause central hypothyroidism (201, 202). However, several investigations suggest that the hypothalamic-pituitary axis is unaffected by these drugs and a specific mechanism has not been discovered (203). Effects appear to be different in relation to the medications considered. For example, in a study evaluating the use of VPA, CBZ and levetiracetam (LEV), thyroid dysfunction was frequent in children taking VPA or CBZ as a monotherapy, but absent in those taking LEV. The authors suggest that, in children with a predisposition for thyroid disease, LEV should be considered over VPA and CBZ, if appropriate for seizure and epilepsy type (204).

7 UNRESOLVED QUESTIONS AND CONCLUSIONS

Various factors, both exogenous and endogenous, can influence and modify the kinetics of the absorption of l-T_4. Pending other targeted studies in the paediatric population, the timing of food intake in relation to l-T_4 administration should always be considered as having the potential to interfere with absorption and parents and other caregivers should be advised to delay the consumption of any food for at least 30, and preferably 60 minutes, after taking l-T_4.

Caregivers, and also healthcare personnel dealing with CH patients, must be informed about possible interactions with food, drugs and the possible influence of diseases on thyroid function and l-T_4 absorption, in order to minimise the negative effects of poorly conducted treatment on the function and development of the central nervous system, especially in the first three years of life.

It is well established that conditions causing malabsorption or reduced gastric acidity can impair the absorption of l-T_4 and the sudden onset of apparently resistant hypothyroidism in patients treated with l-T_4 may be the only clinically observable feature of some of these disorders.

Drugs may interact with TH levels in patients with CH through several mechanisms, influencing thyroid status at the hypothalamic, pituitary, or thyroid level, or affecting the binding of THs to protein carriers and the conversion of T_4 to T_3, as well as the final metabolism and recycling of TH. These drugs may also affect the efficiency of exogenous l-T_4 treatment, requiring vigilance to prevent both undertreatment and overtreatment and to interpret laboratory findings correctly to avoid inappropriate therapeutic changes.

Data about the interference of different foods on l-T_4 absorption in children are very scarce and therefore attention is needed. Liquid formulations are better able to resist interference but all the same, parents and health professionals must remember not to administer l-T_4 therapy too close to food.

8 MAIN CONCERNS

1. CH is one of the most common preventable causes of ID.
2. Newborn CH screening allows early diagnosis and treatment, significantly reducing the risk of irreversible ID and neurologic damage.
3. Clinicians should be aware that certain forms of cCH or milder forms of primary CH may be missed by newborn screening, and it is therefore fundamental that healthcare works are able to recognise the clinical signs and symptoms of CH in order to avoid erroneous or delayed diagnoses.
4. Prompt diagnosis and treatment with adequate doses of l-T_4 leads to excellent neurodevelopmental outcomes in most patients with CH. However, all patients should be closely followed after starting and while under treatment, to monitor their neurological and psychological development.
5. The failure to adequately control CH with oral l-T_4 is a frequent clinical problem.
6. Before increasing the l-T_4 dose in a patient with CH previously well-controlled, it is mandatory to assess adhesion to treatment.
7. In order to avoid the risk of suboptimal care and patient management, it is essential that caregivers and healthcare personnel are aware of the potential effects of food, drugs and diseases on thyroid function and l-T_4 absorption. Possible interferences with l-T_4 treatment, should be re-evaluated at each follow-up visit.
8. l-T_4 oral solution may have a better absorptive profile than tablets and switching from tablet to liquid l-T_4 should be tried before increasing the dose of l-T_4.
9. The risk of overtreatment in the first months of life, and especially in the first 4 weeks, makes it fundamental that patients in this age group receive careful follow-up.

AUTHOR CONTRIBUTIONS

All authors participated in the writing of the manuscript and read and approved the final manuscript.

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Helicobacter pylori Infection Is Associated With Thyroid Dysfunction in Children With Congenital Hypothyroidism

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Helicobacter pylori (*H. pylori*) infection leads to a systemic low-grade inflammatory state and has been associated causally with a diverse spectrum of extra-gastric disorders. Among them, the infection has been involved in the pathogenesis of autoimmune thyroid disease (ATD), but only one study had evaluated children. Therefore, a cross-sectional study was conducted in a cohort of 142 children and adolescents, randomly assessed among those followed up for thyroid diseases in a university pediatric endocrinology service: 106 with congenital hypothyroidism (CH) and 36 with ATD. All children were asymptomatic, under strict control on levothyroxine replacement, and reported no other diseases or use of drugs. *Helicobacter pylori* status was evaluated by the ^{13}C-Urea Breath Test (^{13}C-UBT). Antithyroid antibodies (ATPO, antiTg, and TRAb) and serum thyroid hormones (TSH, free T4, and T3) were assessed by standard assays. Data were analyzed in logistic models by the SPSS statistical software package, and a *p*-value $\leq$0.05 was considered statistically significant. The prevalence of *H. pylori* infection was 19.44% in children with ATD. Neither the gender nor the serum levels of thyroid hormones and antithyroid antibodies were associated with the *H. pylori*-positive status. Thirty-seven (34.90%) children with CH were infected with *H. pylori*. The mean T3 serum level (3.59 $\pm$ 0.84) was significantly lower ($p = 0.001$) in the infected children than in those free from the infection (3.95 $\pm$ 0.89), association that remained after adjustment for the other variables in the multivariate analysis. Because no difference was observed in the levels of TSH and T4, the results indicate that the infection may lead to impairment in the thyroid hormonal balance, but not in the hypothalamic-pituitary-thyroid axis function. In as much as *H. pylori* infection is highly widespread and the prevalence of CH is also not negligible, additional studies are required to confirm our results and to identify the involved mechanisms.

Keywords: *Helicobacter pylori*, congenital hypothyroidism, thyroiditis autoimmune, primary hypothyroidism, childhood, triiodothyronine

INTRODUCTION

Helicobacter pylori (*H. pylori*) is recognized as the main etiological agent of gastritis in human beings and as an essential factor in the pathogenesis of peptic ulcer, gastric carcinoma, and mucosa-associated lymphoid tissue (MALT) type gastric lymphoma (1).

Furthermore, *H. pylori* infection has been associated causally with a diverse spectrum of extra-gastric disorders including iron deficiency anemia, chronic immune thrombocytopenic purpura, growth retardation, and diabetes mellitus (2).

The infection may also be involved in the pathogenesis of autoimmune thyroid disease (ATD), including Hashimoto thyroiditis (HT), and Graves' disease (GD), the major causes of hypothyroidism and hyperthyroidism, respectively (3–6). The mechanism is thought to be linked to molecular mimicry between *H. pylori* antigens and thyroid constituents (4, 6). Furthermore, the association is stronger in patients infected with CagA-positive strains that may be linked to cross-reactivity between antibodies against *H. pylori* CagA protein and follicular cells of the thyroid gland (6). The fact that *H. pylori* eradication leads to decreasing levels of thyroid autoantibodies reinforces the putative role of the infection in ATD (7). However, only one study evaluated *H. pylori* status in children with ATD (8). In addition, we are not aware of studies on *H. pylori* infection in children with other thyroid diseases.

Because the high daily requirement of levothyroxine for the treatment of hypothyroidism has been demonstrated in *H. pylori*-positive adults (9, 10) it could be expected in childhood. Furthermore, in as much as bacterium eradication leads to a reduction of levothyroxine requirement in adults (9, 10), it would also benefit children.

Therefore, our aim was to investigate the association of thyroid dysfunction with *H. pylori* infection in childhood.

MATERIALS AND METHODS

Looking for poor outcomes that could be associated with *H. pylori* infection in childhood, a prospective cross-sectional study was conducted in a cohort of 142 children and adolescents who were randomly assessed. Among them, 106 had congenital hypothyroidism (CH) diagnosed in the public neonatal screening program of the state of Minas Gerais, and 36 children were followed up for thyroid diseases in a Pediatric Endocrinology service of the Hospital das Clínicas - Universidade Federal de Minas Gerais (UFMG). The patients were of similar low socioeconomic level and those with hypothyroidism were under strict control on levothyroxine replacement, according to their bone surface area. They also were asymptomatic in respect to gastric complaints.

Patients with either chronic disease, such as those linked to gastrointestinal absorption alteration, or other autoimmune diseases were not included. The exclusion criteria also included the use of antimicrobial and anti-inflammatory drugs, proton pump inhibitors, and H2 receptor antagonists up to 30 days before the assessment.

The study was approved by the Ethics Committee of the Institution (CAAE-06276712.4.0000.5149) and children, whenever possible, and their legal guardians signed the informed consent.

H. pylori status was evaluated by the ^{13}C-Urea Breath Test (IRIS®, Wagner, Analysen Technik Bremen, Germany). Serum thyroid-stimulating hormone (TSH), free tetraiodothyronine (T4), and free triiodothyronine (T3) were assessed using the ICMA, UniCel® DxI - Beckman Coulter, being the reference value of.34 to 5.6 μUI/ml, 0.54 to 1.24 ng/dl, and 2.5 to 3.9 pg/ ml, respectively. The presence of antithyroid antibodies by antithyroperoxidase (ATPO), antithyroglobulin (antiTg), and anti-TSH receptor (TRAb) was evaluated either by ICMA or ECLIA, Modular E® - Roche, being the reference values of < 9 UI/mL, $< 4{,}11$ UI/mL, and $< 1{,}75$ UI/mL, respectively.

Data were analyzed by the SPSS statistical software package version 20.0 (Chicago, IL, USA). In the logistic models, all the variables with a *p*-value of 0.25 or less in the univariate analyses were included in the full model of logistic regression. Odds ratio (OR) was used as an estimate of the risk. In rare instances when Cornfield estimates of 95% CI were inaccurate, exact limits were calculated instead. The Hosmer-Lemeshow goodness-of-fit test was used to evaluate the fit of the models.

For other comparisons, either the Kolmogorov-Smirnov test or Shapiro-Wilk test based on the number of each sub-group was used to assess the normality of the data. Two-tailed student's t-test or Mann-Whitney U test as well as χ^2 with Yates' correction or Fisher's exact test were employed as indicated. A *p*-value ≤ 0.05 was considered statistically significant.

RESULTS

A hundred and six children had CH (55.7% female, aged 8.79 ± 5.17 years) and 36 had ATD (61.1% female, 13.72 ± 3.72 years old), among them, 27 with HT and 9 with GD.

The prevalence of *H. pylori* infection was of 19.44% in the ATD children. Neither the gender ($p = 0.53$, OR = 0.74, 95%CI = 0.11–5.01), nor the mean ± SD serum levels of TSH (5.52 ± 7.66 vs. 6.61 ± 5.03, $p = 0.41$), T3 (4.27 ± 0.68 vs. 4.27 ± 0.26, $p = 0.40$), T4 (1.20 ± 0.33 vs. 1.29 ± 0.51, $p = 0.66$), ATPO (76.69 ± 97.48 vs. 78.72 ± 101.73, $p = 0.89$), anti-Tg (10.35 ± 12.14 vs. 6.5 ± 9.18, $p = 0.54$), and TRAb (4.40 ± 7.82 vs. 2.43 ± 4.57, $p = 0.41$) was associated with the infection (*H. pylori*-negative vs. *H. pylori*-positive status, respectively). The mean age ± SD was higher ($p = 0.05$) in the *H. pylori*-positive (16.00 ± 3.26;) than in the *H. pylori*-negative (13,15 ± 3.66) children.

Thirty-seven (34.90%) children with CH were infected with *H. pylori* (10.74 mean age ± 4.33 SD) and 69 (65.10%) were *H. pylori*-negative (7.71 mean age ± 5.26 SD). The mean T3 serum level (3.59 ± 0.84) was significantly lower ($p = 0.001$) in the infected children than in those free from the infection (3.95 ± 0.89), association that remained in the multivariate analysis of the logistic model, independently of the other variables (**Table 1**). The mean ± SD levels of ATPO (1.49 ± 0.27), the antiTg (2.61 ± 0.32) and TRAb (0.43 ± 0.28) were in the reference values.

TABLE 1 | *H. pylori*-positive compared with *H. pylori*-negative status in children with CH.

Covariate	Univariate analysis	Multivariate analysis		
	P	OR	95% CI	P
Age	0.002	1.10	0.99–1.20	0.09
Gender	0.46	–	–	–
FT3 pg/mL	<0.001	0.26	0.30–0.98	0.03
FT4 ng/dL	0.30	–	–	–
TSH μUI/mL	0.03	1.05	0.97–1.14	0.25

The Hosmer-Lemeshow test showed a good fit of the model (10 steps; 8 degrees of freedom; p >0.25). CH, Congenital Hypothyroidism; ATD, Autoimmune Thyroid Disease; FT3, free triiodothyronine; FT4, free tetraiodothyronine; TSH, thyroid-stimulating hormone.

TABLE 2 | Host variables associated with CH in comparison with ATD children.

Covariate	Univariate analysis	Multivariate analysis		
	P	OR	95% CI	P
Age	0.002	0.68	0.58–0.79	<0.001
Gender	0.78	–	–	–
HP+	0.11	3.20	1.10–9.60	0.03
FT3 pg/mL	0.13	0.36	0.19–0.67	0.001
FT4 ng/dL	0.48	–	–	–
TSH μUI/mL	0.39	–	–	–

The Hosmer-Lemeshow test showed a good fit (10 steps; 8 degrees of freedom; p >0.25). +, positive; CH, Congenital Hypothyroidism; ATD, Autoimmune Thyroid Disease; FT3, free triiodothyronine; FT4, free tetraiodothyronine; TSH, thyroid-stimulating hormone.

The children did not require an increased daily dose of thyroxine, independent of being *H. pylori*-positive or negative.

When the diseases were compared, the *H. pylori* status, T3 levels, and age were selected in the univariate analysis. In the multivariate analysis, the T3 level (3.82 mean ± 0.88 SD vs. 4.26 mean ± 1.76 SD, respectively) and the age (8.79 mean ± 3.72 SD vs. 13.72 mean ± 5.14 SD, respectively) remained negatively associated with CH and *H. pylori*-positive status positively associated with CH (**Table 2**). The mean levels of ATPO, antiTg, and TRAb were significantly lower in the CH than in the children with ATD ($p < 0.001$ for all).

DISCUSSION

In this cross-sectional study, we evaluated the effects of *H. pylori* infection in children with thyroid disease with an emphasis on CH disease which affects 1:2,000–2,500 newborns (11). There is a consensus that the prevalence of thyroid dysfunction varies according to age, sex, geographical factors, and iodine intake.

Although *H. pylori* infection, especially with CagA-positive strains, has been demonstrated to be associated with ATD in adults (3–6), there is only one study evaluating thyroid disease in children (8). The authors observed an association between the ATD disease and *H. pylori*-positive status, but only concomitantly with the presence of the HLA-DRB1*0301 allele, a genetic marker of autoimmunity. No association was seen in the presence of *H. pylori* positivity/HLA-DRB1*0301 allele negativity as well as *H. pylori* negativity/HLA-DRB1*0301 allele positivity. Therefore, this significant interaction between HLA-DRB1*0301 genotype and *H. pylori* infection supports the role of simultaneous genetic and environmental factors in sustaining the thyroid disease in children. Similarly, we did not observe an association between ATD and *H. pylori* infection in children, considering that the frequency of *H. pylori*-positive status was low, but as expected, it increased with age. Therefore, we may speculate that the role of *H. pylori* in ATD differs between children and adults. In fact, the disease in childhood is at an early stage, and either a longer time of infection or more than one risk factor may be necessary to develop the disease, such as the presence of the HLA-DRB1*0301 allele observed in the study of Larizza and colleagues. The interaction of a genetic and an environmental factor seems to trigger to the development of ATD in childhood (12).

Reinforcing our results, although the hypothesis that the link between *H. pylori* infection and thyroid autoimmunity has been attributed to cross-reactivity between antibodies against *H. pylori* and thyroid constituents, we did not observe differences in the ATPO, anti-Tg, and TRAb levels between the *H. pylori*-positive and -negative children. In a study of the Czech general population (13), the authors observed that persons older than 18 years showed a higher occurrence of antibodies against *H. pylori* and of ATPO, which was not confirmed in the age group younger than 18 years. The lack of association of antibodies with anti-thyroid levels and *H. pylori* infection in children may be due to the fact that it may require a longer time of infection to significantly increase the levels of the autoantibodies.

Because we are unaware of studies evaluating the association between *H. pylori* infection and thyroid dysfunction of other etiology in children, we also evaluated children with CH. To the best of our knowledge, this is the first study demonstrating a high prevalence of *H. pylori* infection in children with CH (34.90%). Although the prevalence of HP infection varies among countries and regions of a country, notably, in the same Brazilian city, Belo Horizonte, a similar *H. pylori* prevalence (37.59%) was demonstrated in a cohort of children submitted to gastroduodenal endoscopy due to gastrointestinal symptoms (14, 15). In another study from northeast Brazil, the *H. pylori* prevalence was reported to be 21.5% in asymptomatic younger children six to 30 months of age (16). Taking together all the data, the differences between children with CH and those with ATD in the present cohort are remarkable. When the diseases were compared, we observed a significantly higher prevalence of *H. pylori* infection among younger children with CH than in those with ATD (19.44 %). It was unexpected because the infection increases with increasing age as we observed in the children with ATD, who were older than those with CH. In addition, it may not be attributed to differences in socioeconomic level.

The significant association between *H. pylori* and CH suggests that the disease could be a predisposing factor to *H. pylori* infection allowing the gastric colonization of the microorganism.

Congenital hypothyroidism is a functional glandular disturbance, due to congenital thyroid dysgenesis or dishormonogenesis (11). Impairment in the development

of the immune system has been demonstrated in mice carrying a mutation (hyt) that results in the phenotypic expression of congenital hypothyroidism (17). Whether some degree of immune response impairment is associated with the human congenital glandular disturbance, it would predispose an increased risk of *H. pylori* infection in childhood.

Furthermore, surprisingly, we observed that serum T3 was lower in *H. pylori*-positive than in *H. pylori*-negative children without differences in the levels of TSH and T4, indicating that the infection leads to an impairment in the thyroid hormonal balance, but not in the hypothalamic-pituitary-thyroid axis function.

Euthyroid Sick Syndrome (ESS) is the term used to identify abnormal thyroid hormone levels in the absence of pituitary or thyroidal dysfunction. According to Chopra et al. (18), the patterns of abnormalities of thyroid hormone levels in ESS are classified into four major types. The most common type, namely low T3 syndrome, was defined as a condition in which T3 is decreased, but T4 and TSH levels remain unaltered as we observed in the group of *H. pylori*-positive children. Variable patterns in serum thyroid hormone concentrations have currently been described as non-thyroidal illness syndrome (NTIS) and may probably occur in any severe illness (19).

Early studies pointed to impaired conversion of the inactive pro-hormone T4 to the biologically active metabolite T3 by the 5-monodeiodinase in the peripheral tissues. The exact cause of changes in thyroid hormone levels observed in NTIS remains controversial and undermined, but would be linked to inflammatory pathways, including cytokine inhibition of the 5-monodeiodinase enzyme. Tumor necrosis factor-alpha (TNF-α) exerts various effects on many cell types. Administration of TNFα to rats decreases hepatic 5'-deiodinase activity (20) and TNF-α has been implicated in the pathogenesis of the low triiodothyronine syndrome in non-thyroidal illness and humans (21, 22). *H. pylori* infection in children is associated with increased inflammatory cytokines release, especially those linked to the innate immune response, among them TNF-α which is increased in children more than in adults with the infection (23).

Of note, in contrast to that observed in adults, the *H. pylori*-positive children with hypothyroidism did not require an increased daily dose of thyroxine. In fact, in adults, impairment of absorption has been associated with an altered acid environment of the stomach due to the infection as well as long-term treatment with proton-pump inhibitors, which are uncommon conditions in children.

Although our study does have some limitations such as the ATD sample size, which could have made the results biased, as well as the incomplete characterization of the bacterium CagA status, the rigorous experimental protocol, and the data analysis strengthen our results. It has to be mentioned that the children were prospectively evaluated and the *H. pylori* status was investigated by a direct test that detects only ongoing infection in contrast to the *H. pylori* infection detection by the presence of antibodies against the bacterium. Of note, serological detection of *H. pylori* antibodies is not useful to discriminate between past and ongoing infections. ^{13}C-UBT, in addition to the stool antigen test, is currently considered the preferred non-invasive method to detect *H. pylori* infection (16, 24). ^{13}C-UBT, besides being validated for Brazilian children, is highly sensitive and specific for the detection of *H. pylori* in children of all ages (16, 24). It is well recognized that children may gain and lose *H. pylori* infection and the serum levels of the antibodies against the bacterium may persist after spontaneous loss.

Taken together, our findings indicate that *H. pylori* infection is not associated with ATD in children, but its prevalence is high in young patients with CH and leads to impairment in the T3 metabolism. In addition, children with hypothyroidism infected by *H. pylori* did not require an increase in the daily dose of thyroxine.

To conclude, we would like to point out that as *H. pylori* infection is highly widespread and the infection is mainly acquired in childhood, in addition to the fact that the prevalence of CH is also not negligible, additional studies are required to confirm our results and to identify the involved mechanisms.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of the Universidade Federal de Minas Gerais (UFMG) – CAAE-06276712.4.0000.5149. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

Conceptualization and writing—original draft preparation: IS, LM, and DQ. Methodology: LM and DQ. Formal analysis: IS and DQ. Writing—review: DQ. All authors contributed to the article and approved the submitted version.

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Primary Congenital Hypothyroidism in Children Below 3 Years Old - Etiology and Treatment With Overtreatment and Undertreatment Risks, a 5-Year Single Centre Experience

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Worldwide neonatal screening for congenital hypothyroidism (CH) is a gold standard of active surveillance in newborns. Prompt diagnosis, subsequent timely treatment implementation, and proper dosage of levothyroxine (L-T4) are crucial for normal growth and development, especially of the central nervous system. However, overtreatment may have a potential negative impact on further neurodevelopment. We retrospectively analysed data of 99 newborns with CH diagnosis, referred to the Endocrinology Outpatient Clinic of the Institute of Mother and Child in Warsaw, Poland from the CH screening program from 2017 to 2021. We evaluated the diagnostic process and treatment up to the age of 3 years. We compared groups of children from the first and the second screening groups (FSG, SSG) in the neonatal screening with an evaluation of ultrasound examination (thyroid dysgenesis vs. gland *in situ*, GIS). The overtreatment and undertreatment risks were assessed and an analysis of the new TSH thresholds was performed. Treatment was implemented at a median of 9 days of life (3 – 27); 8 days (3 – 17) in FSG and 19 (6 – 27) in SSG. The dose of L-T4 differed between FSG and SSG at all three analysed time points (start of the therapy, 12 months, and 3 years) with significantly higher doses in FSG. The same was observed for the patients with thyroid dysgenesis vs. GIS. Screening TSH level was ≥ 28mIU/l in 91.7% of patients with thyroid dysgenesis in comparison to 74.0% of patients with GIS (p= 0.038). The optimally treated group (fT4 in the upper half of the reference range, according to the guidelines) was up to 58.0% of the children during the follow-up. The risk for overtreatment was present in 1/5 of the study group after 12 months and 1/4 after 3 years of L-T4 therapy. Analysis of new TSH thresholds showed an increased prevalence of mild hypothyroidism, GIS, and either euthyroid state or overtreatment while treating with lower L-T4 doses in comparison to the

rest of the cohort. The study confirmed the general efficacy of the CH diagnostic pathway and the timely implemented L-T4 therapy. The suspected overtreatment after the first 12 months of L-T4 therapy requires consideration of the earlier diagnosis re-evaluation.

Keywords: congenital hypothyroidism, permanent CH, transient CH, neurodevelopment, neonatal screening, TSH threshold, CH overtreatment, CH undertreatment

INTRODUCTION

The thyroid hormones (THs) are essential for a harmonious development process in early life. Congenital hypothyroidism (CH) is the most prevalent endocrine disorder in newborns, infants, and children in early childhood, especially below 3 years of age. TH deficiency begins in foetal life due to thyroid axis disturbances. CH may be primary (thyroid gland disorders) or secondary/tertiary (pituitary/hypothalamic disorders); each of them can be temporary (TCH) or permanent (PCH). The incidence of primary CH in the iodine-sufficient areas, which is the topic of this paper, is estimated as 1 in 2000 up to 1 in 3000 live births (1). Currently, we observe an increasing incidence of primary CH, which can be related to the detection of milder cases of CH, and increased survival of preterm neonates (2). In the first years of the screening, the incidence was reported as 1:3000 – 1:4000, but in recent years, it has also been documented as 1:1400 - 1:2800 (3–5).

It is well known that THs play a fundamental role in tissue and organ development in early life. Their role begins in early embryonic life, when in the first half of pregnancy the foetus needs a transplacental transfer of maternal THs. Subsequently the foetal TH production progresses (6). THs optimize critical processes of neurodevelopment and brain maturation, growth and mental development, as well as metabolism (7). In the central nervous system (CNS) the main effects of TH deficiency include deficits in neuronal migration and proliferation, decreased expression of neuronal differentiation factors, reduced cortical thickness, cortical dysplasia, impaired dendrites and axons development, decreased expression of proteins involved in synaptic plasticity, and delayed myelinization (7) and hippocampal development, which is essential for learning and memory functions (6). These complex influences may result in permanent CNS disorders, cause neurological and psychiatric deficits, intellectual disability, spasticity, disturbances of gait and coordination (6), and hearing/attention deficits (8), which cannot be completely resolved with delayed treatment implementation (9).

During the adaptation period in newborns, the THs are also essential for thermogenesis and energy production. THs also influence the cardiovascular system and TH deficiency may cause impaired cardiac diastolic function, increased intima-media thickness, and reduction of exercise capacity and cardiopulmonary function in adult life, despite further proper replacement therapy (10).

The main CH cause worldwide, especially in Africa and Asia, is still iodine deficiency, leading to goitre, deleterious forms of intellectual disability, and in the foetal life – stillbirths or spontaneous abortions, or congenital anomalies (cretinism) (11). However, in the European countries and others with sufficient iodine supply, e.g., in North America, other reasons predominate, among them thyroid dysgenesis (>50%), including athyreosis in 20%-30%, increasing incidence of thyroid dyshormonogenesis (30%-46%), and secondary CH (≈5%) (6, 12–14). In the Polish general population, the adequate iodine intake has been provided due to mandatory iodisation of household salt since 1997 (15).

Thyroid dysgenesis includes agenesis, hypotrophy, hemiagenesis, and ectopy of the thyroid gland. In some cases, especially with family history of CH, gene mutations can be confirmed, including *NKX2-1, NKX2-5, FOXE1, PAX8, TSHR, GNAS, GLIS3, CDCA8, JAG1* genes (2, 6). Thyroid dyshormonogenesis usually is caused by single gene mutations, encoding globulins involved in TH synthesis (thyroid peroxidase - *TPO*, thyroglobulin - *TG*, Na+/I- symporter – *SCL5A5/NIS*, pendrine – *SCL26A4/PDS*), or directly influencing TH synthesis and metabolism (*DUOX2, DUOX2A, IYD/DEHAL1*) (6). The secondary/tertiary CH is the rarest, with an incidence of 1:16,000-50,000, and includes pituitary and/or hypothalamus defects as well as TH resistance (1, 16).

Since the 1970s neonatal screening programs for CH have been implemented worldwide (6). The blood test based on thyreotropin (TSH) concentrations determines primary PCH and TCH and transient hyperthyreotropinemia, but not secondary CH. In Poland, the program started in 1983. The scheme is presented in **Figure 1**. The threshold levels have been lowered since 2012 from TSH ≥35 mIU/ml and TSH 15-35 mIU/ml in the first and second screening, respectively. CH incidence in Poland in the first years of the neonatal screening was documented as 1:3400 – 3500. Based on the screening data, before diagnosis confirmation, the estimated incidence was 1:3888 in Poland and in the region of Warsaw 1:3185 in the years 2017-2021 (unpublished data).

The diagnosis of CH is confirmed by the thyroid function tests (TFTs) and thyroglobulin (TG) analysis and subsequent immediate treatment implementation with oral, solid or liquid, levothyroxine (L-T4). In PCH the treatment will be life-long, but up to 35% of newborns diagnosed with CH reveal to have TCH, which allows for a trial of L-T4 dose reduction or withdrawal after the critical period of neurodevelopment (17).

Timely treatment implementation with a high-dose L-T4 10-15 μg/kg/day initially, within the first two weeks of life, is recommended (1, 6, 9), although optimal L-T4 dosage is still a matter of debate. The main goal is to achieve a rapid TSH normalization and euthyreosis, which have a direct impact on a potentially reversible sequalae of CH (9). On the other hand,

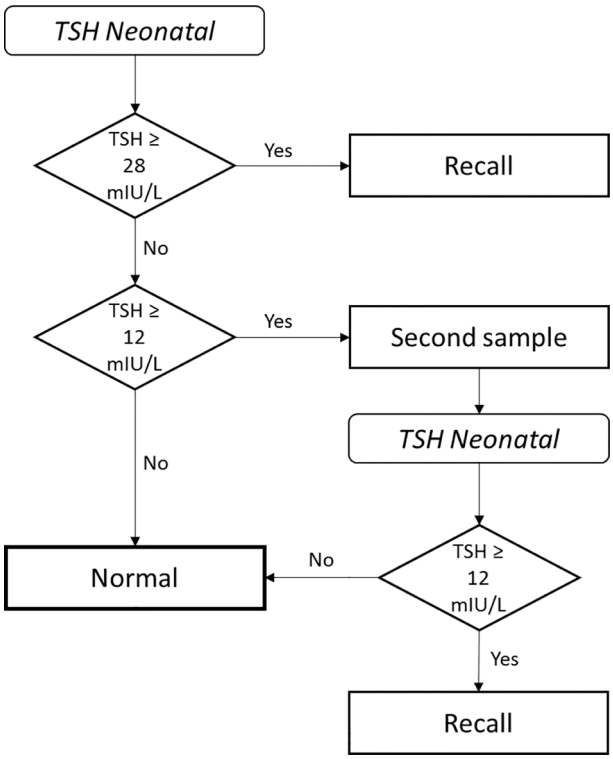

FIGURE 1 | Algorithm for congenital hypothyroidism neonatal screening in Poland.

overtreatment periods in early childhood should also be avoided, as these may cause worse cognitive outcomes in later childhood and adolescence, although the results are not explicit (18–21).

In our study we performed the retrospective analysis of children from the neonatal period up to 3 years of age, screened, diagnosed, and treated in the Institute of Mother and Child, to determine whether there were differences between children from the first and the second screening, as well as between newborns with thyroid dysgenesis and gland *in situ* (GIS). The values of TSH, free thyroxine (fT4) serum concentrations, and L-T4 dosage at the start of the treatment and during the follow up (at 12 months and 3 years) were collected, with particular interest in the treatment strategies in regards to optimal treatment (fT4 level in the upper half of the reference range) or the therapy with initial L-T4 dose below recommended 10 μg/kg/day, and the undertreatment or overtreatment risks. The subgroups of children diagnosed based on the new, decreased TSH threshold values in the neonatal screening and children with a family history of CH were additionally analysed.

MATERIAL AND METHODS

Retrospective analysis of 99 children aged 0-3 years from CH neonatal screening, treated in our clinic between 2017 and 2021, was done. There are seven laboratories involved in CH neonatal screening in Poland, with approximately 330,000-400,000 tests from 380 neonatal departments performed each year. Approximately 1/3 tests from 130 neonatal departments in five regions of Poland is analysed in the Department of Screening and Metabolic Diagnostics in the Institute of Mother and Child in Warsaw and referred to our clinic (22).

In our analysis, the inclusion criteria were referrals from CH neonatal screening. The exclusion criteria were non-CH causes of hypothyroidism. Diagnostic criteria for CH were: TSH >12.40 mIU/l and fT4 <8.37 pmol/l or normal (8.37-22.14 pmol/l); TG <0.9 ng/ml was thyroid agenesis indicator.

L-T4 treatment was implemented in all patients immediately after pediatric endocrinologist consultation, based on the same-day laboratory evaluation of the TFT and TG. All patients received treatment with the solid form of L-T4. Children had a regular follow-up, planned due to the Polish Society for Paediatric Endocrinology and Diabetology recommendations (5). L-T4 dose adjustments were made when applicable. We present the follow-up results at 12 months and 3 years of the therapy with the reference ranges for TSH 0.77 mIU/L - 7.73 mIU/l and fT4 9.44 - 17.70 pmol/l at both time points. Thyroid imaging evaluation was performed during the course of observations, with some delays due to the COVID-19 pandemic. Scintigraphy was postponed for the withdrawal trial after 3 years of life.

The results were analysed separately for the subgroup of children who completed the whole observation period until 3 years of age - complete observation group (COG) and total group of children with CH, including those younger than 3 years or not followed-up in our clinic until the age of 3 years – the general group (GG). The whole cohort was divided into the first screening group (FSG) and the second screening group (SSG), according to the screening process. Screening TSH, TFT, and TG values were examined with a chemiluminescence method – LIAISON XL, DiaSorin.

Statistical Analysis

In statistical analysis, descriptive and inferential statistics were used. The results are presented as means ± standard deviations (SD) for normally distributed data or medians and ranges for non-normally distributed variables. The Kolmogorov-Smirnov test was used for evaluating distribution for normality. Differences between groups were assessed using Student's t-test (or ANOVA) for normally distributed data and non-parametric Mann-Whitney test (or Kruskal-Wallis test) for non-normally distributed parameters. Chi-square test (or Fisher test) was applied to verify hypotheses regarding associations between independent categorical variables. For comparisons between different time points McNemar test was used. A p-value <0.05 was considered to be statistically significant. Statistical analysis was performed using IBM SPSS v. 25.0 software.

RESULTS

All patients were detected by CH neonatal screening program: 79 (79.8%) in the first screening group (FSG), and 20 (20.2%) in the second screening group (SSG). Sixty-one (61.6%) children were in the COG subgroup. Patient profiles at birth are presented in **Table 1**.

The First vs. the Second Screening Group

TSH concentrations at the start of treatment differed between FSG and SSG: ranged from 7.3 to 345 mIU/l and from 10.7 to 100 mIU/l, respectively. The median values are not provided, and the upper ranges can be biased, because part of the laboratory confirmatory tests provided only results up to the upper limit of a diagnostic method (TSH >100 mIU/l), with no further dilution analysis.

There was no association between screening TSH level and gestational age, gender, Apgar score, or birth length. However, birth weight SDS <-2 was more frequent in newborns from SSG than FSG (15.0 vs. 1.3%, p=0.014).

Thyroid Dysgenesis vs. Gland *in situ*

In 86 (86.9% of GG) children examined with thyroid ultrasound, GIS was found in 50 (58.1%) and thyroid dysgenesis in 36 (41.9%) patients (**Table 2**). Among dysgenesis: agenesis was detected in 26 (30.3%), ectopy in two (2.3%), hemiagenesis in one (1.2%), and hypoplastic thyroid gland in seven (8.1%) cases.

Screening TSH ≥28 mIU/l was detected in 91.7% patients with dysgenesis in comparison to 74.0% patients with GIS (p=0.038). All patients with thyroid agenesis and ectopy had screening TSH concentrations ≥28 mIU/l. In the group of patients with dysgenesis, the proportion of girls was higher than in the group with GIS (72.2 vs. 50.0%, p=0.039).

TG serum concentration was examined in 72 (72.7%) newborns; in six (8.3%) the TG level was <0.9 ng/ml (**Table 1**); in four of them the thyroid imaging was performed: agenesis was detected in three and GIS in one patient. The median TG concentration was significantly decreased in patients with dysgenesis in comparison to the patients with GIS, 85.8 ng/ml (0.0 – 2242.0) vs. 210.2 ng/ml (0.19 – 4816.0), (p=0.002), respectively. Among patients with dysgenesis, newborns with agenesis presented the lowest TG concentration.

Treatment with Levothyroxine

We did not observe an association between TSH at the start of the treatment and birth data, gender, or thyroid dysgenesis diagnosed by ultrasonography, but there was an association with the start of the treatment day. The results of TFTs, TG, and L-T4 dose at the start of treatment, 12 months and 3 years are presented in **Table 3**. In girls fT4 at the start of the therapy was within the reference range in 36.7% and below the range in 63.3% and in boys in 63.2% and 36.8%, respectively (p=0.010). In girls the mean fT4 at the start of the treatment was 7.15 ± 5.05 pmol/l, in boys – 9.66 ± 5.36 pmol/l (p=0.022); there was no such

TABLE 1 | Patient profiles at birth.

	Total (n = 99)	FSG (n = 79)	SSG (n = 20)	p-value
girls (n,%)	61 (61.6%)	51 (64.6%)	10 (50.0%)	0.232
TSH1, mIU/l (mean ± SD)	102.8 ± 65.6	123.9 ± 56.2	19.3 ± 4.5	<0.001
TSH2, mIU/l (mean ± SD)	23.4 ± 11.1	x	23.4 ± 11.1	x
gestational age, median (range)	39 (25-43)	39 (25-43)	39 (31-41)	0.239
Apgar score, median (range)	10 (4-10)	10 (4-10)	10 (6-10)	0.502
birth weight SDS (mean ± SD)	-0.27 ± 1.11	-0.22 ± 0.94	-0.48 ± 1.64	0.353
birth length SDS (mean ± SD)	2.10 ± 1.11	2.18 ± 1.06	1.81 ± 1.39	0.198
dysgenesis (n,%)	36 (41.9%)	33 (47.1%)	3 (18.8%)	0.038
GIS (n,%)	50 (58.1%)	37 (52.9%)	13 (81.3%)	
family history (n,%)	20 (20.2%)	13 (16.5%)	7 (35.0%)	0.065
TG <0.9 ng/ml (n,%)	6 (8.3%)	6 (10.3%)	0 (0.0%)	0.342

FSG, first screening group; SSG, second screening group; GIS, gland in situ.

TABLE 2 | Ultrasonography evaluation in the examined group.

	Total (n = 86)	Dysgenesis (n = 36)	GIS (n = 50)	p-value
Girls (n,%)	51 (%)	26 (72.2%)	25 (50.0%)	0.039
TSH1, mIU/l (mean ± SD)	103.0 ± 64.9	126.0 ± 59.7	86.5 ± 64.1	0.009
TSH2, mIU/l (mean ± SD)	24.2 ± 12.3	18.7 ± 5.3	25.5 ± 13.2	0.364
Gestational age, median (range)	39 (25-42)	39 (34-42)	39 (25-41)	0.755
Apgar score, median (range)	10 (4-10)	10 (6-10)	10 (4-10)	0.015
Birth weight, g, median (range)	3400 (940-4310)	3395 (1870-4310)	3415 (940-4300)	0.707
Birth weight SDS (mean ± SD)	-0.23 ± 1.13	-0.28 ± 1.05	-0.21 ± 1.20	0.780
Birth length, cm, median (range)	54 (37-60)	55 (46-60)	54 (37-60)	0.563
Birth length SDS (mean ± SD)	2.13 ± 1.14	2.23 ± 1.21	2.05 ± 1.09	0.453
TSH1 ≥28 mIU/l (n,%)	70 (81.4%)	33 (91.7%)	37 (74.0%)	0.038
Family history (n,%)	16 (18.6%)	1 (2.8%)	15 (30.0%)	0.001
TG <0.9 ng/ml (n,%)	4 (6.6%)	3 (12.0%)	1 (2.8%)	0.296

GIS, gland in situ; TSH1, TSH in the first screening; TSH2, TSH in the second screening.

association observed with TG, and the associations were not confirmed in further observations. We found an association between fT4 at the start of the treatment with thyroid dysgenesis – mean fT4 was significantly lower than in GIS (p=0.010). TSH levels were lower in males during the follow-up – median TSH at 12 months was 2.58 mIU/l in boys vs. 3.11 mIU/l in girls (p=0.002) and at 3 years 1.77 mIU/l vs. 2.37 (p=0.006), respectively. There was no association between the diagnostic results of the initial fT4 or TG and the start of the treatment day.

Treatment was implemented at median of 9 days of life (3 – 27): in 8 days (3 – 17) in FSG and 19 days (6 – 27) in SSG (p<0.01) (**Figure 2**). There was no association between the start of the treatment day and TFTs at 12 months and 3 years, nor TG level. In three patients from FSG the therapy started above the recommended 14th day of life (16 - 17), and in 14 children in the SSG group (15 - 27). Eleven of these children had normal fT4 levels at the start of the treatment.

The dose of L-T4 in GG was significantly higher in the patients from FSG compared to SSG at all three analysed time points, as presented in **Table 3**. Similar observations as in GG applied to COG subgroup: mean L-T4 starting dose in FSG was 11.1 ± 3.1 μg/kg/day and in SSG – 7.7 ± 4.1 μg/kg/day (p=0.001). During the follow-up at 12 months mean L-T4 dose in FSG was 4.1 ± 1.1 μg/kg/day and in SSG – 3.2 ± 1.0 μg/kg/day (p=0.009), at 3 years of age mean L-T4 dose in FSG was 3.6 ± 1.1 μg/kg/day and in SSG – 2.2 ± 0.9 μg/kg/day (p<0.001).

We found an association at every time point in GG between L-T4 dose and thyroid dysgenesis (**Table 4**). This relationship was confirmed also in the COG: at the start of treatment – in dysgenesis mean L-T4 dose was 11.3 ± 3.2 μg/kg/day and in GIS – 9.39 ± 3.4 μg/kg/day (p=0.030), and at 3 years - in dysgenesis

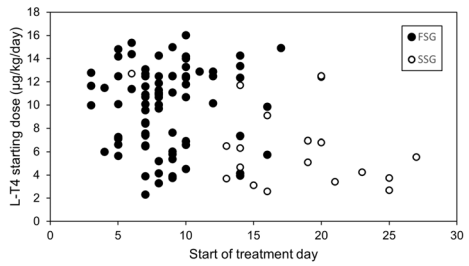

FIGURE 2 | Start of the treatment day and levothyroxine starting dose in the first (FSG) and the second (SSG) screening groups.

TABLE 3 | Thyroid function test results and levothyroxine treatment at the start of the treatment, after 12 months and 3 years.

		Total (n = 99)	FSG (n = 79)	SSG (n = 20)	p-value
Start of the treatment (n=99)	fT4, pmol/l (mean ± SD)	8.1 ± 5.3	7.4 ± 5.2	10.9 ± 4.8	0.009
	L-T4 dose, μg/kg/d (mean ± SD)	9.3 ± 3.8	9.9 ± 3.5	6.8 ± 3.7	0.002
12 months (n=89)	TSH, mIU/l (median, range)	2.8 (0.02-82.6)	3.1 (0.02-82.6)	1.9 (0.1-13.0)	0.319
	fT4, pmol/l (mean ± SD)	16.6 ± 6.5	16.8 ± 7.1	15.8 ± 1.9	0.579
	L-T4 dose, μg/kg/d (mean ± SD)	4.0 ± 1.4	4.2 ± 1.4	3.2 ± 0.9	0.013
3 years (n=61)	TSH, mIU/l (median, range)	2.0 (0.02-20.2)	2.4 (0.02-20.2)	1.8 (0.04-9.4)	0.538
	fT4, pmol/l (mean ± SD)	18.1 ± 3.7	18.3 ± 3.7	17.3 ± 3.6	0.363
	L-T4 dose, μg/kg/d (mean ± SD)	3.3 ± 1.2	3.6 ± 1.1	2.2 ± 0.9	<0.001

FSG, first screening group; SSG, second screening group.

TABLE 4 | Levothyroxine treatment and thyroid ultrasonography evaluation.

		Total (n = 86)	Dysgenesis (n = 36)	GIS (n = 50)	p-value
Start of the treatment	fT4, pmol/l (mean ± SD)	8.0 ± 5.4	6.2 ± 4.4	9.2 ± 5.8	0.010
	L-T4 dose, µg/kg/d (mean ± SD)	9.4 ± 3.7	11.0 ± 3.4	8.3 ± 3.5	0.001
12 months	TSH, mIU/l (median, range)	2.6 (0.02-82.6)	3.1 (0.03-23.9)	2.5 (0.02-82.6)	0.922
	fT4, pmol/l (mean ± SD)	16.7 ± 6.7	17.6 ± 9.2	16.1 ± 3.5	0.322
	L-T4 dose, µg/kg/d (mean ± SD)	4.0 ± 1.4	4.4 ± 1.5	3.7 ± 1.3	0.035
3 years	TSH, mIU/l (median, range)	2.0 (0.02-20.2)	1.9 (0.02-16.2)	2.3 (0.07-20.2)	0.462
	fT4, pmol/l (mean ± SD)	18.2 ± 3.7	18.6 ± 3.3	17.8 ± 4.2	0.398
	L-T4 dose, µg/kg/d (mean ± SD)	3.3 ± 1.2	3.7 ± 1.1	2.9 ± 1.3	0.017

GIS, gland in situ.

mean L-T4 dose was 3.7 ± 1.1 µg/kg/day and in GIS – 2.9 ± 1.3 µg/kg/day (p=0.017); such association was not observed at 12 months. We did not find an association in any of the time points between L-T4 dosage and TSH at the start of the treatment.

In the analysis of the therapeutic goals, we identified the group of patients with the optimal treatment according to European Society of Paediatric Endocrinology (ESPE) guidelines (2), ENDO-European Reference Network (ENDO-ERN) Consensus (1), and the Polish Society for Paediatric Endocrinology and Diabetology recommendations (9), defined as fT4 level in the upper half of the reference range during follow-up. At 12 months, 51 (58%) out of 88 patients (27 girls), and at 3 years 27 (44.3%) out of 61 patients (19 girls) were treated optimally. In this group, there were 22 (46.8%) patients with dysgenesis at 12 months and 12 (50.0%) at 3 years, compared to the rest of the group - 14 (41.2%) and 20 (58.8%), respectively. In the optimally treated group vs. the rest of the group mean L-T4 dose was 3.9 ± 1.5 µg/kg/day vs. 4.1 ± 1.3 µg/kg/day (p=0.625) at 12 months and 2.8 ± 1.1 µg/kg/day vs. 3.7 ± 1.2 µg/kg/day (p=0.005) at 3 years.

Additionally, we also analysed a group receiving L-T4 starting dose below recommended 10 µg/kg/day (2.60 - 9.86 µg/kg/day). There were 47 (47.5%) newborns in this group: 32 (40.5%) in FSG and 15 (75%) in SSG (p=0.006). At the start of the treatment mean fT4 was 9.7 ± 5.7 pmol/l compared to 6.7 ± 4.5 pmol/l in a group treated with the recommended L-T4 dose (p=0.005). The median start of the treatment day was 9 days (4 – 27) and in the rest of the cohort – 8 days (3 – 20) (p=0.038). In the follow-up at 12 months median TSH was 3.40 mIU/l vs. 1.93 mIU/l in the group receiving the recommended L-T4 starting dose (p=0.034); mean fT4 was 17.00 ± 8.78 pmol/l vs. 16.31 ± 3.63 pmol/l, respectively (p=0.163). At 3 years there were no such associations observed. In 38 patients with available ultrasound examination, GIS predominated – 29 (76.3%).

Overtreatment and Undertreatment Risks

The analysis of overtreatment and undertreatment data is presented in **Table 5**. We divided 88 patients tested at 12 months and 61 tested at 3 years into three groups defined as (1) euthyreosis (TSH N, fT4 N) or isolated fT4 elevation (TSH N, fT4↑); (2) overtreatment: sub-hyperthyreosis (TSH↓, fT4 N) or hyperthyreosis (TSH↓, fT4↑); (3) undertreatment: sub-hypothyreosis (TSH↑, fT4 N) or hypothyreosis (TSH↑, fT4↓). We included patients with the isolated fT4 elevation (eight patients, 9.1% at 12 months and 16, 26.2% at 3 years) to the euthyreosis group, as it can be attributed to compliance issues.

The euthyreosis was observed in 54 patients (61.4%) at 12 months and in 39 patients (63.9%) at 3 years of the follow-up, and there was no association between treatment status and FSG and SSG groups. At both time points the group of overtreated predominated over the undertreated patients: at 12 months 19 (21.6%) vs. 15 (17%), at 3 years 15 (24.6%) and seven (11.5%), respectively. At 12 months there was also a substantial group

TABLE 5 | Overtreatment and undertreatment in follow-up after 12 months and 3 years.

		Total (n = 99)	FSG (n = 79)	SSG (n = 20)	p-value
12 months (n,%)	Euthyreosis	54 (61.4%)	42 (59.2%)	12 (70.6%)	0.799
n=88	Overtreatment	19 (21.6%)	16 (22.5%)	3 (17.6%)	
	Undertreatment	15 (17.0%)	13 (18.3%)	2 (11.8%)	
3 years (n,%)	Euthyreosis	39 (63.9%)	30 (62.5%)	9 (69.2%)	1.000
n=61	Overtreatment	15 (24.6%)	12 (25.0%)	3 (23.1%)	
	Undertreatment	7 (11.5%)	6 (12.5%)	1 (7.7%)	

FSG, first screening group; SSG, second screening group.

with hypothyreosis, which diminished at 3 years time point. In the overtreatment group, mean starting L-T4 dose was 10.7 ± 2.6 µg/kg/day, 4.2 ± 0.8 µg/kg/day after 12 months, and 3.6 ± 1.1 µg/kg/day at 3 years. We did not find an association between the treatment status and FSG/SSG, the start of the treatment day, gender, or GIS in ultrasonography.

Additionally, we compared patients' treatment status between time points 12 months and 3 years. Data are presented in **Figure 3**. We observed that among patients followed up for 3 years (COG) the proportions of euthyreosis were similar at both time points – 38 (62.3%) vs. 39 (63.9%), the hyperthyroidism group also remained similar – 16 (26.2%) vs. 15 (24.6%), and seven (11.5%) patients had hypothyroidism at both time points.

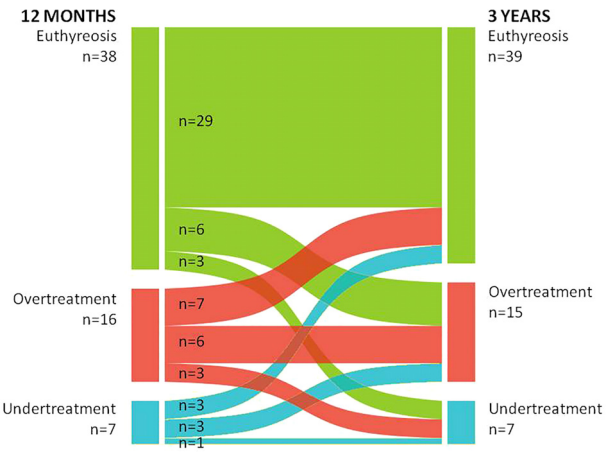

FIGURE 3 | Comparison of the treatment status at 12 months and 3 years.

Seven patients did not change treatment status – six remained in overtreatment and one in undertreatment group.

New TSH Threshold Values

We analysed the data of two subgroups of patients, who were qualified for CH based on the new screening threshold range, recommended in Poland since 2012, with screening TSH values of 12 - 15 mIU/l (n=3) and 28 - 35 mIU/l (n=7). The results are presented in **Table 6**.

We did not find differences between these groups and the rest of the cohort regarding the birth characteristics. Interestingly, three patients (42.9%) from the group with TSH 28 - 35 mIU/l had a family history of CH, compared to 10 patients (13.9%) with screening TSH ≥35 mIU/l, but also in the group with TSH 12 - 15 mIU/l there was one child with a positive family history. The frequency of dysgenesis was comparable in both groups, one and two patients, respectively.

All the children in the group with TSH 12 - 15 mIU/l had a good treatment outcome in terms of TFTs results during the follow-up. This effect was achieved with relatively lower L-T4 doses. Similarly, in the group with TSH 28 - 35 mIU/l, most children had TSH within the normal reference range during the follow-up. However, more patients showed higher fT4 values.

Family History of Congenital Hypothyroidism

In our cohort, 20 children (20.2%), including 11 girls (55%), had a family history of CH; having an older sibling with CH. Interestingly, they were present in both FSG (n=13, 16.5% of GG) and SSG (n=7, 35% of GG), but with a tendency toward a higher risk in the SSG (p=0.065). Among the patients with available ultrasonography evaluation, only one patient had thyroid agenesis, and 15 children presented GIS. There were no differences in the TFTs during the follow-up, except surprisingly higher fT4 value at the start of the therapy

TABLE 6 | Initial characteristics, thyroid function tests, and levothyroxine dosage in subgroups in relation to the previous TSH threshold values.

		12-15mIU/l (n = 3)	15-28mIU/l (n = 17)	28-35mIU/l (n = 7)	≥35mIU/l (n = 72)	p-value
Dysgenesis (n,%)		1 (33.3%)	2 (15.4%)	2 (28.6%)	31 (49.2%)	0.121
GIS (n,%)		2 (66.7%)	11 (84.6%)	5 (71.4%)	32 (50.8%)	
Family history (n,%)		1 (33.3%)	6 (35.3%)	3 (42.9%)	10 (13.9%)	0.081
TG <0.9 ng/mL (n,%)		0 (0.0%)	0 (0.0%)	0 (0.0%)	6 (11.5%)	0.508
Start of the treatment (n = 99)	fT4, pmol/l (mean ± SD)	12.4 ± 2.5	10.6 ± 5.2	14.6 ± 1.6	6.7 ± 4.9	<0.001
	L-T4 dose, µg/kg/d (mean ± SD)	3.9 ± 1.0	7.3 ± 3.7	6.7 ± 3.3	10.2 ± 3.4	<0.001
12 months (n = 89)	TSH, mIU/l median (range)	2.8 (1.6-3.4)	3.6 (0.1-13.0)	2.8 (0.02-16.0)	3.4 (0.02-82.6)	0.796
	fT4, pmol/l (mean ± SD)	14.8 ± 3.6	16.0 ± 1.4	16.8 ± 2.3	16.8 ± 7.4	0.940
	L-T4 dose, µg/kg/d (mean ± SD)	2.5 ± 0.7	3.4 ± 0.9	2.9 ± 1.1	4.3 ± 1.4	0.003
3 years (n = 61)	TSH, mIU/l median (range)	1.4 (1.0-1.8)	2.0 (0.04-9.4)	1.3 (0.5-20.2)	2.6 (0.02-16.2)	0.872
	fT4, pmol/l (mean ± SD)	14.5 ± 3.1	17.8 ± 3.5	17.2 ± 3.2	18.5 ± 3.8	0.450
	L-T4 dose, µg/kg/d (mean ± SD)	1.3 ± 1.8	2.4 ± 0.6	3.1 ± 1.1	3.7 ± 1.1	0.001

compared to the patients with no CH family history (11.18 ± 6.11 vs. 7.34 ± 4.80 pmol/l, p=0.003). L-T4 dose was lower at the start of the treatment and at 3 years of follow-up (6.91 ± 3.56 vs. 9.86 ± 3.58, p=0.001 and 2.18 ± 1.54 vs. 3.44 ± 1.12 μg/kg/day, p=0.014, respectively). This difference was also shown for six patients with the positive family history in COG.

DISCUSSION

The pathogenesis of CH is complex and it is well known that it can be influenced by environmental and genetic factors. The genetic background was not examined in our study. The risk factors for neonatal CH, transient, or permanent vary between countries and include gender, premature birth, worse newborn health status, and children born as small for gestational age (SGA) (23–26). In our cohort mean fT4 level at the start of treatment was lower in girls, but despite it, no significant difference was observed in the initial L-T4 dose. Among newborns with thyroid dysgenesis, females were predominant (72%), and that was also observed by the other authors (27, 28).

Thyroid dysgenesis remains the most common cause of CH, although dyshormonogenesis frequency seems to be rising (6, 14). However, in our study among children examined with thyroid ultrasound, GIS was found in most cases (58.1%), but the newborns with dysgenesis (both agenesis and ectopy) predominated in FSG. As expected, we found lower mean fT4 at the start of the treatment in patients with thyroid dysgenesis compared to GIS. It was reflected also in the initial L-T4 dosage which was higher in children with dysgenesis, which allows predicting the increased risk of the life-long need for the treatment in these patients (29).

It is still discussed whether the screening TSH can predict the transient or permanent forms of CH. The important question is if the screening TSH test can be useful to select the patients who could be treated less extensively to avoid overtreatment and reduce parents' anxiety and medical care costs (30–32). It is known from other data that screening TSH tends to be lower in neonates with TCH than in those with PCH (17, 33–35). In our study, decreased screening TSH results were observed in newborns with birth weight below -2 SDS who are mostly burdened with TCH risk. Therefore early (3 up to 5 days of life) determination of TSH and fT4 concentration in the serum in these groups of children, regardless of the CH screening program, is recommended in Poland (9, 36).

Di Dalmazi et al. showed that neonatal TSH can be influenced by gender: male newborns had higher TSH concentrations than females, and by gestational age: preterm newborns had lower TSH values than the term ones (34, 37). In our study, we also observed a tendency toward lower screening TSH in preterm newborns. In contrast, according to Bosh-Gimenez et al., TSH concentrations were found to be higher in neonates born with SGA (38).

The results of TG values in the studied cohort are not explicit and there is a need for further evaluation of this particular examination. TG is essential for TH synthesis and homozygous or compound heterozygous mutations of the TG gene can result in PCH (39). Most often TG concentration is increased in patients with dyshormonogenesis. Although most patients with CH due to TG gene mutations show decreased serum TG levels (40), in some rare cases elevated serum TG was observed (39). In our study, median TG was decreased in newborns with dysgenesis in comparison to GIS, as expected the lowest TG concentrations were detected in newborns with agenesis. In our opinion, patients with positive results for TG and absent thyroid gland in ultrasonography need to be the first-line group for the thyroid scintigraphy, due to the possibility of the ectopy. However, also in the patients with CH and GIS, with possible dyshormonogenesis, the scintigraphy can add additional diagnostic value, as these patients may have an iodine uptake/organification defect (12). In our study, the goiter was not observed in the examined group, but according to the recent concept, the patients with CH and GIS may present some form of dyshormonogenesis despite not presenting the classical goitrous form of CH (14).

According to the recommendations, treatment for CH should be started as soon as possible, within 14 days of life, with no delay for diagnostic procedures (1, 6, 9). In our study, the treatment predictably started earlier and with higher L-T4 doses in FSG than in SSG. In patients with delayed treatment implementation, the reason was mainly due to extended screening procedures (SSG) and technical problems with the patients' arrival for endocrine evaluation. However, most of these patients had normal fT4 levels at the start of the treatment.

The goal of the treatment is to obtain and maintain fT4 levels within the upper half of the reference range (1, 6, 9). In our study, this group consisted of 58.0% and 44.3% of children in subsequent time points, and we did not find any association with other analysed factors like birth data, gender, or ultrasonography evaluation. We suspect that the most important factors to obtain the optimal treatment requirement are compliance in terms of method of L-T4 administration, daily adherence, meal time distance, avoiding foods that influence L-T4 absorption (i.e., soy formula, calcium or iron supplements), and families understanding of the critical importance of the regular therapy (9, 29). Also isolated fT4 elevation may be attributed to compliance issues related to taking the L-T4 dose shorter than 4 hours before blood sampling or chronic poor adherence with "making up" by taking multiple doses before the scheduled test and endocrine consultation (29). However, also with optimal compliance, some patients seem to require higher L-T4 doses to normalize TSH values, which may suggest a form of "T4 resistance" (21, 29).

At the 12 months time point the mean L-T4 dose in the SSG was 3.2 μg/kg/day, which according to ENDO-ERN consensus, could suggest TCH in patients with GIS and is close to the rationale for the need for the treatment re-evaluation after 6 months of age (recommended L-T4 dose <3 μg/kg/day) (1). Also, the differences in L-T4 dosages between FSG and SSG groups in both follow-up time points supported the above data. Although the median L-T4 doses in both groups at 12 months and 3 years were lower than established in Polish recommendations (3) the

disease control in terms of TFTs results showed a higher risk for over- than undertreatment. The undertreatment was present in 17% of the study group at 12 months and 11.5% at 3 years. Only one patient remained undertreated both at 12 months and 3 years. In other analysed studies undertreatment prevalence was 12/61 (19.6%) (19), 24/55 (46%) (20), and 11/88 (13%) (21).

According to a German group, episodes of undertreatment in the first 3 months of life led to increased scores in the Withdrawn, Anxious, Social, and Thought (WAST) test used for an autism diagnosis. Also, children with severe CH undertreated at 3-6 months of age were more likely to have anxiety and introversion social problems compared to the rest of the group (20).

In respect to decreasing threshold values in neonatal screening and subsequently including in the treatment the newborns with mild CH, mostly with GIS confirmed in ultrasonography evaluation, the question arises if high-dose L-T4 treatment strategy should not be reserved for the patients with severe primary CH (3–5, 14). In our study, we assessed separately the group treated with the starting L-T4 dose of <10 µg/kg/day. It was a relatively large group of 47 children (47.5%) with mean fT4 within the reference range, and 76.3% of these patients with available ultrasonography examination had GIS, which can be attributable to TCH. The rate of TCH is established as 7% – 35% in different studies (10, 17). Analysis of other TCH predictors in German and Austrian databases, apart from GIS, were TSH at the start of the treatment <73 mIU/l and median L-T4 dosage 3.1 µg/kg/day at 12 months and 2.9 µg/kg/day at 2 years (17).

There is an extensive discussion regarding proper L-T4 dosing in respect to the long-term neurocognitive outcome in children with CH, how to keep the beneficial effect of prompt and effective treatment, and to avoid risks of side effects at the same time. Currently recommended starting L-T4 dose often (up to 60% of patients) leads to overtreatment (8, 16, 29). In our study, the overall overtreatment rate was 21.6% after 12 months and 24.6% after 3 years, with L-T4 doses not exceeding the recommended range. Frequent follow-up at the beginning of the treatment warrants the proper dose adjustments. Moreover, ESPE and ENDO-ERN recommendations state that, in case of obtaining TFTs with elevated fT4, dose reduction should not be based on a single result, unless evident TSH suppression (1, 6).

The overtreatment episodes may have clinical implications, although the data are inconsistent. A Spanish study, where 50 children with CH were observed, revealed a relation of the number of overtreatment episodes in the first 6 months with alertness deficits at school age – inability to maintain focus of attention or respond appropriately and resist inappropriate behaviours (18). A German study based on an analysis of 61 children with CH up to 11 years suggested that overtreatment during the first 2 years had a subtle negative impact on cognitive development, compared to undertreatment (19). In another study of the above group, with an evaluation of 55 patients, the association of the Attention, Delinquency, and Aggression (ADA) score (used in the diagnosis of attention deficit hyperactivity syndrome, ADHD) with overtreatment in 1 – 3 months of life was reported (16). However, in the meta-analysis performed by Aleksander et al., including 438 patients, the authors pointed out that the recommended L-T4 dosage is safe and efficient, and lower doses can lead to 6-8 points lower IQ in children with severe CH (21). In our study, the group with overtreatment was more frequent than undertreatment at both time points of the follow-up. In this group, six patients who were overtreated at 12 months, were also overtreated at 3 years of age.

The last decade brought a wide discussion regarding the notable increase in the incidence of CH in regard to changing the TSH thresholds criteria in many screening programs throughout the world (3–5, 14, 35, 40). It has been observed in many studies that the lowering of TSH screening values has enabled clinicians to identify more children with suspected CH. However, part of this group most probably represents either mild or TCH. It has been discussed whether the treatment of these patients is indeed necessary.

In our cohort, we observed similar results, after lowering the TSH threshold values in the two subgroups of patients qualified for treatment based on revised, lowered screening criteria. Mainly mild hypothyroidism with the mean fT4 values within the reference range and GIS was present. Moreover, after the 12 months and 3 years of L-T4 treatment, the TFTs showed either euthyroid state or overtreatment while treated with lower L-T4 doses in comparison to the rest of the cohort. Most authors recommend treating the patients with the milder form of the disease after fulfilling the changed screening criteria, at least in the first years of life. This approach seems to be appropriate even if the therapy influence on the neurocognitive function in these milder cases, especially with normal fT4 results at the start of the treatment, has not been fully evaluated yet (3, 4, 35). In borderline cases of mild hypothyroidism, there is a possibility to consider the LT4 treatment withdrawal after 12 months (6, 41). However, the Polish Society for Paediatric Endocrinology and Diabetology recommends performing the re-diagnostic process after the 3 years of life (9). This re-evaluation is planned in our study group after 3 years of life, together with the thyroid scintigraphy examination.

Regarding CH family history, surprisingly we found only one case of thyroid dysgenesis in this group. Moreover, fT4 at the start of the treatment was higher in this group compared to the rest of the cohort. This again may implicate a wide pathogenesis background of CH, with the possible dyshormonogenesis background associated with milder CH. Therefore, genetic studies, especially in families with more than one child affected, are needed.

Our study has its limitations, mainly limited imaging diagnostic examinations, without the thyroid scintigraphy, that could have explained in more detail the pathogenesis of CH in the presented children. We were not able to present the genetic background of the patients as well. Moreover, a lack of precise actual TSH levels following dilution at the diagnosis is a significant limitation of the initial data analysis. The strengths of the research consist of a relatively large group of patients followed up in a single centre with one diagnostic and therapeutic approach to the children with CH. Three points of

control were selected for the analysis to decrease the vast amount of data. Further evaluation of more detailed observation, especially in between the visits during the first year of life, is planned.

CONCLUSIONS

The analysis of the 5-year experience in the single centre confirms the general efficacy of the CH diagnostic pathway and the timely implemented L-T4 therapy. The imaging examinations, thyroid ultrasound, or even more sufficient thyroid scintigraphy, seem to have a significant role in the diagnostic and therapeutic process in CH, as higher L-T4 doses could be recommended to patients with thyroid dysgenesis.

However, the results show a relatively significant risk for overtreatment, presented in 1/5 of the study group after 12 months and 1/4 after 3 years of the L-T4 therapy. The suspected overtreatment after the first 12 months of L-T4 therapy requires more detailed observation with consideration of the diagnosis re-evaluation process. Together with a recent more frequent diagnosis of mild hypothyroidism in newborns, clinicians may consider an earlier trial of lowering the L-T4 dose and possible withdrawal of the treatment. The recommended frequency of clinical appointments should be respected to detect and prevent the over- and undertreatment episodes in children with CH during the follow-up.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

All authors have accepted responsibility for the content of the manuscript and approved submission. EL contributed to the plan of the study, prepared basic materials, took part in data collection, wrote and revised the manuscript. AL-A contributed to the plan of the study, wrote and revised the manuscript. DW contributed to data collection, wrote and revised the manuscript. KS, EM performed the statistical analysis of the study and revised the manuscript. MO contributed to up-to-date data of the neonatal screening program.

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Treating Thyroid Associated Ophthalmopathy in Pediatric Patients

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Thyroid associated ophthalmopathy (TAO) is a common extra-thyroid clinical manifestation of Graves' disease. It is an inflammatory disease of the eye and orbital tissues. Up to one-third of pediatric Graves' disease patients could be diagnosed with TAO. The symptoms can be variable with remissions and exacerbations of pediatric Graves' disease, which has negative effects on the quality of life in children. Teprotumumab is a fully human IgG1κ type monoclonal antibody targeting insulin-like growth factor-1 receptor (IGF-1R), and was approved for the treatment of TAO as a "breakthrough therapy" by the FDA in 2020. Nevertheless, the safety and effectiveness have not been established in pediatric patients. IGF-1R plays an important role in human development, which raises concerns of developmental toxicity. As presented in the pharmacology review report, juvenile monkeys were tested in two separate repeated-dose toxicity studies and no NOAEL was identified. Teprotumumab affected the growth, thymus, spleen and decreased the bone growth. Younger animals seemed to be more sensitive to the effects on normal growth and normal thymus. Hearing impairment posed additional risk to the potential pediatric use, especially for school-age children. Considering the nature of the target, Teprotumumab should not be used empirically in children. More efforts would be made for the further development of teprotumumab for pediatric use.

Keywords: thyroid associated ophthalmopathy, pediatrics, IGF-1R, teprotumumab, children

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INTRODUCTION

Either adult or child patients with Graves' disease could suffer from the ophthalmic thyroid associated ophthalmopathy (TAO), but obviously few attentions were paid for the latter population. Up to one-third of pediatric Graves' disease patients could be diagnosed with TAO (1). The symptoms can be variable with remissions and exacerbations of primary disease (2), which seriously affects the quality of life. Currently, glucocorticoid is the first-line therapy for pediatric TAO, but the efficacy was controversial and there could be risks for severe adverse reactions (3, 4). Activation of insulin-like growth factor-1 receptor (IGF-1R) signaling and overexpression of IGF-1R in the orbital fibroblasts, B cells and T cells were reported in Graves' disease patients (5). Teprotumumab is a fully human IgG1κ type monoclonal antibody targeting IGF-1R, and is originally indicated for cancer treatment. The antibody specifically binds to IGF-1R and blocks its activation and signaling.

The drug was approved for the treatment of TAO as a "breakthrough therapy" by the FDA in 2020. Nevertheless, the safety and effectiveness have not been established in pediatric patients.

CONCERNS ABOUT USING TEPROTUMUMAB IN CHILDREN

Considering the nature of the target, teprotumumab should not be used empirically in children. Despite of the proven elevation of IGF-1R in adult TAO patients, pediatric data are scarce. Whether there might be any differences in the etiology involved IGF-1R on TAO between adults and children are largely unknown. Besides, IGF-1R plays an important role in human development (6), which raises concerns of developmental toxicity. Actually, as presented in the pharmacology review report, juvenile monkeys were tested in two separate repeated-dose toxicity studies (both 13 weeks) and no NOAEL (no observed adverse effect level) was identified (7). Teprotumumab affected the growth, thymus, spleen and decreased the bone growth. Younger animals seemed to be more sensitive to the effects on normal growth and normal thymus. Moreover, literature has also documented the functions of IGF-1R in the developmental reproduction and central nervous systems (8–10). Although it is not clear whether teprotumumab could affect these systems, attention can be paid in future studies. Other common adverse actions of teprotumumab indicated for TAO include infusion reactions, exacerbation of preexisting inflammatory bowel disease, muscle spasm, nausea, alopecia, diarrhea, fatigue, hyperglycemia, hearing impairment, dry skin, dysgeusia and headache (11). Most of which are considered manageable based on monitoring and intervention. Recently, there are increasingly case reports on the ototoxicity of teprotumumab (12–14). Even though current data indicate that most of the otologic symptoms resolved after treatment, hearing loss may be persistent in adults (15). Hearing impairment posed additional risk to the potential pediatric use, especially for school-age children.

DISCUSSION

Observational studies exploring the expression of IGF-1 and IGF-1R in children with TAO may help to verify the therapeutic potential of teprotumumab. Further toxicity studies using juvenile animals are particularly important for exploring the developmental effects and obtaining toxicological threshold doses (e.g. NOAEL) in order to set the basis for the design of initial dose for pediatric use. Mechanism studies could be conducted to confirm the clinical significance when necessary. If neither safe doses nor acceptable developmental toxicity might be observed in additional juvenile toxicology studies, it would be recommended to find out the youngest age for a pediatric TAO patient to receive teprotumumab therapy safely based on all available data. Changing the current route of administration of teprotumumab (iv) to topical administration (e.g. periorbital injection) may be an alternative to reducing systemic toxicity. Clinical trials are essential and an evidence-based risk-benefit assessment should be performed. If the results cannot reach the endpoints, more efforts would have to be made to discover new targets and develop novel medicines for treating TAO in pediatric patients.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

XW contributed to the conception of the work and reviewed the manuscript; TD drafted and reviewed the manuscript; ZF made critical revisions to the manuscript. All authors contributed to the article and approved the submitted version.

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The prevalence of hypothyroxinemia in premature newborns

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Congenital hypothyroidism diagnosed by TSH assessment in bloodspot screening may be overlooked in preterm newborns due to immaturity of the hypothalamus-pituitary-thyroid axis in them. The purpose of the study was to determine the prevalence and causes of hypothyroxinemia in preterm newborns, determined by TSH and FT4 serum concentration measurement, performed on the 3-5th day of life. We assessed TSH, FT4 and FT3 serum concentration on the 3-5th day of life in preterm children born at our centre within three consecutive years. We assessed the incidence of hypothyroxinemia, and its cause: primary hypothyroidism, secondary hypothyroidism or low FT4 syndrome - with normal TSH concentration, its dependence - among others - on gestational age (GA), birth body weight (BBW) and being SGA. A total of 525 preterm children were examined. FT4 concentration was decreased in 14.9% of preterm newborns. The most frequent cause of hypothyroxinemia was low FT4 syndrome (79.5%). More than 92% cases of hypothyroxinemia occurred in children born before the 32nd week and/or with BBW below 1500 g. Thus, every fourth child in these groups had a reduced FT4 concentration. Neonates with hypothyroxinemia were significantly lighter than those with normal FT4. In older and heavier neonates with hypothyroxinemia, serious congenital defects were observed. Neither IVH nor SGA nor twin pregnancies predispose children to hypothyroxinemia. Among newborns with untreated hypothyroxinemia in whom TSH and FT4 assessment was repeated within 2-5 weeks, a decreased FT4 concentration was confirmed in 56.1% of cases. As hypothyroxinemia affects 25% of newborns born before the 32nd week of gestation and those in whom BBW is less than 1500g, it seems that in this group of children the newborn screening should be extended to measure serum TSH and FT4 concentration between the 3-5th day of life. In older and heavier

neonates, additional serum TSH and FT4 assessment should be limited to children with severe congenital abnormalities but not to all SGA or twins. Despite the fact that the most common form of preterm hypothyroxinemia is low FT4 syndrome, it should be emphasized that FT4 remains lowered on subsequent testing in more them 50% of cases.

KEYWORDS

congenital hypothyroidism, hypothyroxinemia, neonatal screening, preterm newborns, small for gestational age, thyroid hormones, thyroid stimulating hormone

Introduction

Thyroid hormones (free thyroxine – FT4 and triiodotyronine - FT3) play an important role in the growth and maturation of many cells and tissues, not only in the foetal but also in the neonatal period. They are essential for the normal development of the brain (1, 2).

Congenital hypothyroidism is defined as a dysfunction of the hypothalamic–pituitary–thyroid axis, present at birth and resulting in insufficient thyroid hormones synthesis (3). Therefore, this condition may be caused by abnormal development or function of the hypothalamus, pituitary gland or thyroid gland and it should be detected immediately after birth, and treated with levothyroxine (LT4) in order to avoid irreversible developmental disorders (3). For many years, screening for congenital hypothyroidism has been performed around the world to diagnose primary hypothyroidism by assessing TSH in a dry blood specimen (from a heel prick), collected between the 3rd and 5th day of life. As secondary hypothyroidism is overlooked in this way, when financial resources are available it is recommended to measure FT4 to screen for central (i.e. secondary, due to insufficient secretion of TRH or TSH) hypothyroidism (3). While the frequency of secondary hypothyroidism is rare in full-term newborns, it is more frequent in preterm infants as the hypothalamic–pituitary–thyroid axis starts to mature by the second trimester of gestation (4–6). Therefore, the more preterm the child is, the more immature this axis is and no specific feedback regulation is observed. Thus, in these cases, both the lack of increased TSH secretion as in primary hypothyroidism and decreased TRH and TSH secretion, resulting in insufficient FT4 secretion (secondary hypothyroidism) due to the immaturity (a transient form) or disorders of the hypothalamus and pituitary gland (a permanent form), were observed (4–6).

The risk factors for hypothyroxinemia – besides prematurity – included low body birth weight (BBW), twin pregnancy, critical illness or medication during pregnancy (3, 7). Many studies discuss the validity of assessing TSH and FT4 levels in all premature babies (7, 8). In Poland, screening has been carried out for many years, involving the assessment of TSH in a drop of blood from a heel prick. So additional recommendations were published in Poland (2016) due to the need for early diagnosis and treatment of hypothyroidism in preterm newborns (9, 10). These recommendations point to the need to measure TSH and FT4 serum concentrations on the 3rd-5th day of life (regardless of the National Newborn Screening Programme of inborn metabolism) in all preterm children, to initiate treatment with LT4 before the end of the second week of life (in case of hypothyroxinemia confirmation). According to the TSH values accompanying the reduction in FT4 concentration, primary hypothyroidism, secondary hypothyroidism or the low FT4 syndrome were diagnosed and treatment with an appropriate dose of LT4 was recommended. Therefore, we have started to implement these recommendations at our Institute, which is hospital of the 3rd level of reference for taking care of newborns and provides regional and supraregional services.

Recently, the Consensus Guidelines Update was published by the European Society for Pediatric Endocrinology (ESPE) and European Society for Endocrinology (ESE). They recommend considering post-screening procedures in special categories of neonates at the risk of congenital hypothyroidism, including premature, with low birth weight, twins and sick babies (3). However, it is not clear whether all preterm and SGA neonates should be screened additionally for hypothyroxinemia, or who and when should be treated with LT4.

Thus, the purpose of the study was to determine the prevalence and causes of hypothyroxinemia in premature and SGA neonates born at the Polish Mother's Memorial Hospital - Research Institute (PMMH-RI) in Lodz in the last three years.

Material and methods

At the PMMH-RI in Lodz, in three (3) consecutive years: 2019, 2020 and 2021, the concentrations of TSH, FT4 and FT3 in

the blood serum were assessed on the 3rd-5th day of life, regardless of the screening test (assessment of TSH in a dry blood specimen) in children born preterm (GA <37 weeks). We collected the following data: GA, BBW, total APGAR score, single or twin pregnancy, intraventricular haemorrhage (IVH), serious congenital defects, severe illness required Intensive Care Units (ICU) and the causes of child's death during primary hospitalisation (if it happens).

To analyze hypothyroxinemia frequency depending on GA, we divided a group of children into the following subgroups:

A: younger than 24 weeks,
B: $24_{0/7}$ to <28 weeks,
C: $28_{0/7}$ to <32 weeks,
D: $32_{0/7}$ to <37 weeks.

In addition, to analyze the concentration of FT4, FT3 and TSH, depending on BBW, we divided the children into the following subgroups:

ILBW - incredible low birth weight - BBW <750 g,
ELBW - extremely low birth weight - BBW ≥750 g and < 1000 g,
VLBW - very low birth weight - BBW ≥1000 g and <1500 g,
LBW - low birth weight - BBW ≥ 1500 g and < 2500 g,
NBW – normal birth weight - BBW >2500g.

The concentrations of TSH, FT4 and FT3 were measured using the electrochemiluminescent immunoassays (ECLIA) method with commercially available appropriate kits (Roche Diagnostic, Mannheim, Germany). Normal range values were as follows: for TSH: 0.7–12.0 mIU/l, with inter-assay coefficients of variation (CVs) 1.3–1.8%, for FT4: 0.89–2.2 ng/dl; and for FT3: 1.95-6.03 pg/ml with CVs 2.0–2.4%.

In accordance with Polish recommendations, we divided our group of premature babies into the following diagnostic groups (9, 10):

1. primary hypothyroidism (PHT) - if hypothyroxinemia was accompanied with elevated TSH concentration (≥12 uIU/ml);
2. secondary hypothyroidism (SHT) - if hypothyroxinemia was accompanied with reduced TSH concentration (<0.7 uIU/ml);
3. low FT4 syndrome - if hypothyroxinemia was accompanied with normal TSH concentration (≥ 0.7 uIU/ml and <12 uIU/ml).

All children with hypothyroxinemia were consulted by one of pediatric endocrinologists (all of them are co-authors of present study). According to their recommendations, in some cases, LT4 treatment was started immediately, while in others, it was recommended to repeat the test within 2-5 weeks and to take a therapeutic decision depending on the next results. L-thyroxine was recommended in doses depending on the diagnosis: in PHT – 10-15 ucg/kg, in SHT – 7-10 ucg/kg and in low FT4 syndrome: 3-7 ucg/kg (9, 10).

The data were analyzed using Statistica 11.0 software (StatSoft, Inc., Tulsa, OK, USA). The continuous variables were expressed as mean ± standard deviation for normally distributed variables. Shapiro-Wilk's test was used to test the distribution of the variables. Correlations were evaluated using the Pearson's test while the comparison of some count data was performed using the chi-square test. A one-way ANOVA was applied for statistical analysis with the subsequent use of a *post-hoc* test, in order to statistically assess differences between groups; Tukey's test was selected because of the uneven amount of data in individual groups. $p < 0.05$ was accepted as significant value.

The diagnosis of PHT has been confirmed in all the children by a typical screening test from heel. Due to the very high concentration of TSH and very low FT4 and FT3 (the results are shown in Table 1), those cases were excluded from the comparative analyses concerning TSH, FT4 and FT3 results.

Results

The prevalence of hypothyroxinemia in preterm newborns

A total of 525 preterm newborns (246 female and 279 male) were examined. In 78 (14.9%) of them, FT4 concentration was

TABLE 1 Mean (± SD) values of TSH, FT4 and FT3 concentrations in newborns with hypothyroxinemia in individual subgroups, according to TSH values concentration.

	Primary hypothyroidism	Secondary hypothyroidism	Low FT4 syndrome	P=
No of children	3	13	62	
TSH (uIU/ml)	56.35 ± 42.69	0.47 ± 0.20	3.17 ± 2.09	0.000000
FT4 (ng/ml)	0.38 ± 0.18*	0.59 ± 0.15	0.62 ± 0.15*	0.032
FT3 (pg/ml)	0.69 ± 0.51	1.04 ± 0.40	1.23 ± 0.40	0.056

Data marked with (*) differ by p <0.01.

decreased, while in 447 (85.1%) - it was within normal range. The group of children with hypothyroxinemia included 34 females (43.6%) and 44 males (56.4%). Among all preterm children, a low FT4 was confirmed in 13.8% of female and 15.8% of male children. The chi^2 test results indicate that there were no statistical differences as regards the incidence of a decreased FT4 concentration among preterm girls and preterm boys.

The impacts of gestational age on the occurrence of hypothyroxinemia in preterm newborns

We analyzed the occurrence of hypothyroxinemia in the group of 525 premature infants in relation to their GA. The results are shown in (Figures 1A–D). The incidence of reduced FT4 secretion was inversely proportionate to the child's gestational age (Figures 1A, B).

We found that in the group of children born before the 24th week of GA, the incidence of hypothyroxinemia was as high as 71.4%, among those born between the 24th and 28th week – 53.1%, between the 28th and 32nd week – 9.2%, while between the 32nd and 37th week – only 2.1% (five cases) (Figures 1C, D).

Thus, the analysis of all preterm children born before the 32nd week (n=288) showed that hypothyroxinemia occurred in 73 (25.3%) of them. That means that every fourth child in that group had hypothyroxinemia. When we analysed children born between the 32nd and 37th week (n=234), hypothyroxinemia was found only in 5 (2.1%) of them (chi^2 = 40.76, p<0.00005). To conclude, 93.6% cases of hypothyroxinemia were observed in children born before the 32nd week of GA.

The impact of birth body weight on the occurrence of hypothyroxinemia in preterm newborns

We also analyzed the occurrence of hypothyroxinemia in the group of 525 preterm newborns, depending on their BBW. The results are shown in Figures 2A, B. The incidence of decreased FT4 secretion was inversely proportionate to the child's birth weight. We found that in the group of children born with a BBW of less than 750 g, the incidence of hypothyroxinemia was as high as 64.3%, in those with BBW between ≥750 g and <1000 g - it was 41.1%, while in those with BBW between ≥1000 g and <1500 g, it was only 10.3%. Among children with BBW between ≥1500 g and <2500 g, reduced FT4 was observed in only a few (six) cases (2.7%), while among babies weighing more than 2500g - in none. Thus, 92.3% of all cases of hypothyroxinemia were observed in children born with

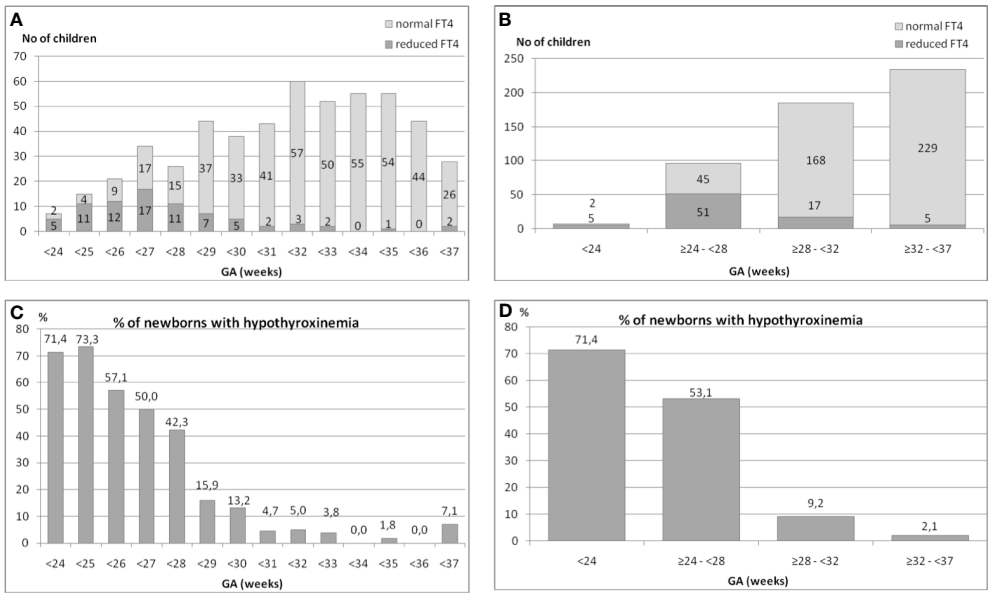

FIGURE 1
(A–D). The incidence of hypothyroxinemia and normal FT4 concentration in the group of 525 preterm infants in relation to their gestational age (GA).

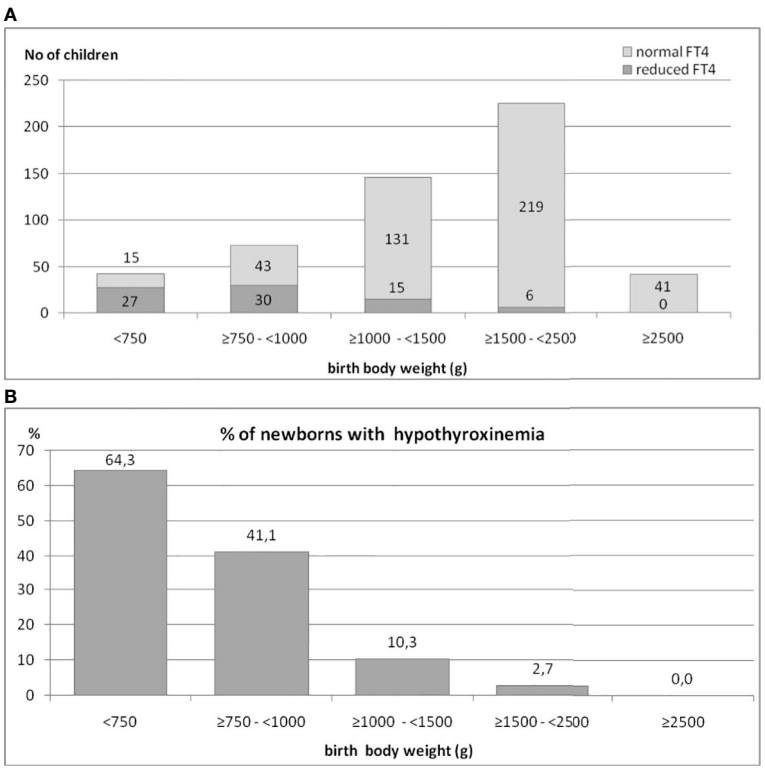

FIGURE 2
(A, B). The occurrence of hypothyroxinemia in the group of 525 preterm newborns, depending on their birth body weight.

BBW below 1500 g (72 cases of hypothyroxinemia out of 189 children), while in children born with BBW ≥1500 g, hypothyroxinemia was observed in 6 out of 260 children (chi^2 = 67.3, p<0.0001). In the group of babies born with BBW below 1500 g, the incidence of hypothyroxinemia was 27.6%.

The impact of being SGA on the occurrence of hypothyroxinemia in preterm newborns

We investigated how many preterm children were born with SGA in the group of children with normal FT4 and with hypothyroxinemia. We found that in the group with hypothyroxinemia, only 5 out of 78 had been born as SGA, however, the incidence was slightly higher than in the group of preterm children with normal FT4 – 12 out of 447 (2.7%), chi^2 = 2.94, p=0.08 (see Table 2).

We also compared the mean BBW in individual subgroups (in GA ranges), in children with hypothyroxinemia and those with normal FT4 concentration. We found that for group B: $24_{0/7}$ to <28 weeks, and for group C: $28_{0/7}$ to <32 weeks, children with hypothyroxinemia were significantly lighter (Table 3).

Hypothyroxinemia was associated with a lower BBW of the child, but in many cases, the birth body weight was not low enough to meet the SGA criterion (Table 3).

TABLE 2 The incidence of SGA, AGA and LGA among children with hypothyroxinemia and with normal FT4 concentration (SGA, small for gestational age; AGA, appropriate for gestational age; LGA, large for gestational age; SDS, standard deviation score).

Birth weight	Hypothyroxinemia, n=78	Normal FT4, n=447	
SGA (BW SDS <-2.0)	5 (6.4%)	12 (2.7%)	Chi^2 = 2.94; P=0.08
AGA (BW SDS ≤+2.0 and ≥-2.0)	66 (84.6%)	402 (89.9%)	Chi^2 = 1.94; P=0.16
LGA (BW SDS >+2.0)	7 (9.0%)	33 (7.4%)	Chi^2 = 0.24; P=0.62

TABLE 3 Mean (± SD) values of body birth weight (BBW) and BBW SDS and in individual subgroups (according to GA) of children with hypothyroxinemia and those with normal FT4 concentration.

GA (weeks)	<24	≥24 and <28	≥28 and <32	≥32 and <37
No of children	5/2	51/44	17/178	5/232
BBW in children with hypothyroxinemia (g)	604.0 ± 100.39	776.0 ± 164.80	1047.64 ± 315.68	2098.0 ± 501.27
BBW in children with normal FT4 (g)	590.0 ± 56.56	931.0 ± 199.47	1358.45 ± 356.17	2030.26 ± 487.77
P=	0.864	0.000007	0.0006	0.759
BBW SDS in children with hypothyroxinemia	1.7 ± 1.82	0.47 ± 1.29	-0.32 ± 1.12	0.06 ± 1.91
BBW SDS in children with normal FT4	1.45 ± 1.03	1.25 ± 1.32	0.46 ± 1.16	0.03 ± 1.20
P=	0.864	0.004	0.008	0.952

The impact of being a twin on the occurrence of hypothyroxinemia in preterm newborns

The analysed group of preterm children included 96 twins: 8 out of 78 children (10.3%) with hypothyroxinemia and 88 out of 447 children (19.7%) with normal FT4 concentration. Thus, the incidence of twin pregnancies was higher in the group of newborns with normal FT4 concentration than in the one with hypothyroxinemia (chi^2 = 0.21; p>0.05).

The impact of intraventricular haemorrhage on the occurrence of hypothyroxinemia in preterm newborns

Intraventricular haemorrhage (IVH) was found in 30 children with a decreased FT4 concentration, but in most of them, it was a low grade haemorrhage: grade I - in 4 children and grade II - in 17 children. Serious IVH occurred in 9 newborns: grade III - in 6 children and grade IV - in 3 children. In 6 of those babies, the low FT4 syndrome was diagnosed (9.7% of the entire low FT4 subgroup), and in 3 - SHT was confirmed (23.1% of the entire SHT group). So, the occurrence of serious IVH was the most frequent cause of SHT, however the chi^2 test result for these values was not statistically significant (chi^2 = 1.83, p>0.05). Nevertheless, we found that the mean TSH concentration did not differ between children with IVH and without it: 2.1 ± 1.87 uIU/ml vs 2.78 ± 2.19 uIU/ml, respectively, but the mean FT4 concentration was significantly lower in children with IVH than in children without this complication (0.52 ± 0.22 ng/ml vs 0.63 ± 0.14 ng/ml, respectively, p <0.05).

An analysis of children with hypothyroxinemia born after the 32nd week and with a BBW over 1500g

The detailed data on the five children with hypothyroxinemia who were born between the 32nd and 37th week (and one who was born in the 30th week, but with the body mass of over 1500 g), are presented in Table 4. In all those children, we observed some serious congenital defects.

TABLE 4 Detailed data on five children with hypothyroxinemia, born between 32nd and 37th week and one child born in the 30th week, but with the birth weight over 1500 g (GA, gestational age; BBW, birth body weight; SDS, standard deviation score; SHT, secondary hypothyroidism; GDM, gestational diabetes mellitus).

Case	sex	GA (weeks)	BBW (g)	BBW SDS	FT4 (ng/ml)	FT3 (pg/ml)	TSH (mIU/l)	Type of disorder	Apgar score	death	LT4 treatment	Comorbidity
C1	f	30	1600	1.2	0.69	0.99	8.78	Low FT4 syndrome	7/8	no	no	gastrointestinal perforation
C2	f	32	1600	0.1	0.6	1.23	1.74	Low FT4 syndrome	5/6	no	no	congenital hydrocephalus, polymicrogyria
C3	f	32	2350	2.3	0.82	1.25	5.03	Low FT4 syndrome	5/7	no	no	dilated pulmonary trunk, GDM and Hashimoto's disease - in mother
C4	m	34	2750	1.5	0.66	1.38	0.58	SHT	4/5	yes	no	cardiomyopathy, polyhydramnios, dysmorphic features
C5	m	36	1590	-2.5	0.23	0.54	0.32	SHT	7/8	no	yes	oligohydramnios, kidney failure
C6	m	36	2200	-1.0	0.71	2.14	4.37	Low FT4 syndrome	6/7	no	yes	agenesis of the corpus callosum, colpocephaly, cerebral hypoplasia

The causes of hypothyroxinemia in preterm newborns

Depending on the TSH concentration result, among 78 children with hypothyroxinemia, 3.8% were diagnosed with PHT (n = 3), 16.7% - SHT (n = 13), and 79.5% - with the low FT4 syndrome (with normal TSH concentration) (n = 62). In all children with PTH, the diagnosis was also made on the basis of typical neonatal TSH screening (from a heel prick). The results of TSH, FT4 and FT3 levels in individual groups are presented in Table 1. Apart from the obvious difference in TSH concentration, which was a precondition for the division into groups, it was noticed that FT4 concentration was significantly lower in the PHT subgroup than in the low FT4 syndrome subgroup (Table 1).

The causes of death among preterm newborns with hypothyroxinemia

In the analyzed group of children with reduced FT4 concentration, 8 died shortly after delivery (10.3%), 2 of them were treated with LT4, while the remaining children were not. Secondary NT was diagnosed in five (5) newborns, and low FT4 syndrome in three (3) of them. Thus, among the children with SHT, death occurred in 5 out of 13 newborns (38.5%), while among those with the low FT4 syndrome - in 3 out of 62 (4.8%). In none of those children hypothyroxinemia was the cause of death; they died due to serious concomitant illnesses.

The usefulness of FT3 assessment in the diagnosis of hypothyroidism in preterm newborns

In addition to TSH and FT4, we also evaluated the concentration of FT3 at the same time point. Among 78 children with hypothyroxinemia, low FT3 concentration was found in 67 newborns (85.9%), normal - in 8 newborns (10.3%) and in 3 of them (3.8%) this parameter was not assessed. We found low FT3 concentrations in all children with SHT (except one), in 52 out of 62 newborns with the low FT4 syndrome (in 7 - it was normal and in 3 - it was not measured), as well as in all newborns with PHT. We did not find any differences as regards FT3 concentration among groups (Table 1).

We observed a strong positive correlation between FT4 and FT3 ($r=0.7$, $p<0.05$) in the whole group of the analysed children.

Among 447 newborns with normal FT4 concentration, low FT3 concentration was confirmed in 36 babies (8%). In three of them, also the TSH level was low and in those cases FT4 concentration was in the lower limit of normal range.

Therefore, it seems that FT3 measurement does not add additional information in terms of assessing the secretion of TSH and FT4 axes in newborns.

The prevalence of hypothyroxinemia in subsequent tests performed within 2-5 weeks in preterm newborns not treated following decreased FT4 in the first test

In 30 out of 71 children with hypothyroxinemia who were alive (seven babies died within the first 6 weeks of life, and one - included in this analysis - died in the 13th month of age), LT4 therapy was started immediately (including 3 children with PHT), while in the rest of them, the test was repeated within 2-5 weeks. In 23 out of 41 children (56.1%), the FT4 level was still decreased in the second measurement and LT4 therapy was ordered. In 18 out of 41 (43.9%) cases, normalisation of FT4 concentration was observed and children were not treated (Figure 3). We found no difference in the initial levels of FT4, FT3 or TSH, or GA and BBW between the groups of children with and without subsequent improvement in FT4 levels (with transient or persistent hypothyroxinemia) (Table 5).

Discussion

In our study, we assessed serum TSH and FT4 levels shortly after birth, in premature infants, born over the period of three years at the PMMH-RI, in order to contribute to the discussion of whether serum TSH and FT4 assessments are worth running in addition to the routine TSH screening for all premature newborns, for the purpose of detecting hypothyroxinemia and initiating LT4 treatment as soon as possible, to avoid serious consequences related to disorders in the nervous system development (1, 2).

In 2016, based on their best knowledge, available at that time, a group of Polish experts established rules of conduct which we implemented at our centre. The main goal was to secure the proper development of the premature infants' nervous system with the appropriate amount of LT4. Although the number of reports of transient hypothyroxinemia in premature infants and considerations regarding the principles and indications for initiating LT4 treatment is increasing, universal rules have not yet been established. Some recommendations propose that the assessment should be limited to TSH and FT4 in children born before the 32nd week of gestation and with a birth body weight (BBW) lower than

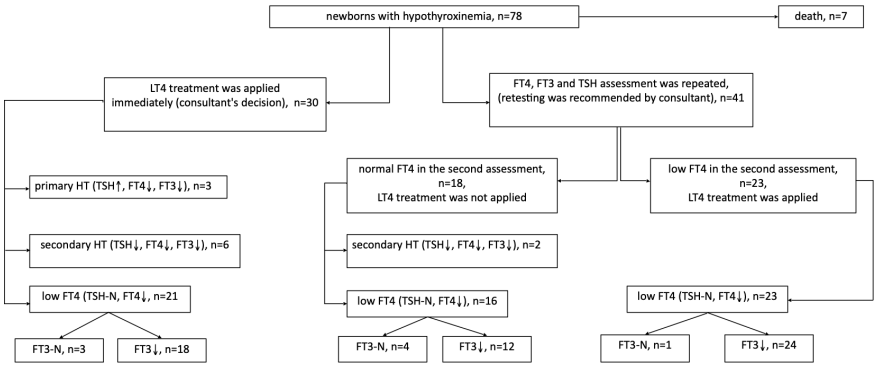

FIGURE 3
Follow up of children with hypothyroxinemia depending on the decision regarding LT4 treatment, taking into account the cause of the disease.

1500 g, also in critically ill children or children from twin pregnancies (3, 11, 12).

We examined such newborns and found that the problem affected a large group (15%) of premature babies. However, on the basis of further analysis, it turned out that those were mainly children born before the 32nd week of pregnancy and with a birth weight below 1500 g. Among those children, the incidence of hypothyroxinemia was as high as 24.6% (born before the 32nd week) and 27.6% (born with BBW lower than 1500 g), comprising 92.5% of all the cases with hypothroxinemia that we detected. Therefore, we would recommend running an additional screening in that group of newborns. A similar recommendation was given by others (11–14).

In order to search for the cause of hypothyroxinemia and to establish the principles of LT4 therapy, we divided our group of children with reduced FT4 concentration into 3 subgroups, depending on the TSH result. This division was also based on our Polish recommendations, published in 2016 (9, 10). This was important because, depending on the TSH result, a different LT4 dose is recommended and, at the same time, it indicates the likelihood of the persistent or transient form of hypothyroxinemia.

However, it is debatable whether the limits of TSH are well matched and whether the distinction between secondary HT and the low FT4 syndrome is logical.

Our analysis shows that out of the three possible causes of hypothyroidism, the low FT4 syndrome is most commonly (79.5%) represented. All the cases of primary hypothyroidism were detected in a standard TSH screening and therefore did not constitute a clinical problem.

Out of 13 cases of secondary hypothyroidism, 5 children died shortly after birth, which means that their condition was very severe. Thus, it is difficult to determine whether the low TSH levels accompanying hypothyroxinemia were actually caused by secondary hypothyroidism or were due to a critical illness. This is an important issue, because the syndrome of low thyroxine and triiodothyronine in severely ill patients is not treated, and in the case of secondary hypothyroidism in premature newborns, LT4 replacement therapy is recommended. In this group, as many as 5 (out of 13) had severe IVH and those children did die - however, it did not provide a satisfactory answer to the question whether low

TABLE 5 Mean (± SD) values of screening TSH, FT4 and FT3 serum concentrations in the group of preterm newborns with hypothyroxinemia, depending on the initial therapeutic decision and the results of tests performed thereafter (three PHT children were excluded from the analysis).

	LT4 treatment was applied immediately	FT4, FT3 and TSH assessment was repeated within 2-5 weeks		P=
		reduced FT4 in the second assessment	normal FT4 in the second assessment	
No of children	27	23	18	
TSH (uIU/ml)	2.30 ± 1.82	3.15 ± 1.97	2.84 ± 2.33	0.339
FT4 (ng/ml)	0.61 ± 0.17	0.60 ± 0.16	0.66 ± 0.14	0.505
FT3 (pg/ml)	1.19 ± 0.44	1.20 ± 0.38	1.29 ± 0.44	0.316
GA (weeks)	26.70 ± 2.82	25.83 ± 2.66	27.56 ± 2.23	0.119
BBW (g)	895.00 ± 395.96	796.09 ± 327.63	965.00 ± 274.02	0.296

FT4 was caused by damage to the hypothalamic-pituitary axis or a serious illness of the newborn (15, 16).

The added value of our work compared to others was the assessment of the concentration of FT3, together with TSH and FT4. We wondered if this might help to distinguish the low FT4 syndrome caused by the child's severe condition from the immaturity of the axis. However, the concentrations of FT4 and FT3 correlated positively with each other, and there were no differences between the concentrations of FT3 in individual groups of diagnoses.

Despite the reports in the literature on a higher incidence of hypothyroxinemia in children from twin pregnancies, we did not confirm that observation in the analysed group of children (17, 18), perhaps due to small number of cases.

On the other hand, the reports on the higher incidence of hypothyroxinemia in children with SGA (9, 19) were also unconfirmed in our study. In turn, in the group of preterm newborns with hypothyroxinemia, there was a slightly higher prevalence of children born as SGA (6.4%) than in the group of preterm newborns with normal FT4 levels (2.7%). However, it was the lower birth weight that predisposed the children from the different gestational age groups to having low FT4. Thus, it was being leaner (malnourished) rather than being SGA that was a hypothyroxinemia risk factor. The data provided by Kaluarachchi et al. (20) did not confirm the higher incidence of congenital hypothyroidism in neonates with SGA after considering potential confounding factors. However, they mentioned that TSH levels were higher in newborns with SGA time compared to newborns without SGA. A similar observation was reported by Grob et al. (21) and Uchiyama et al. (22) - they emphasized that being SGA is strongly associated with delayed higher TSH, also in children with SGA born with BBW below 2000 g.

As it has been mentioned above, 92.3% of hypothyroxinemia cases are children born before the 32nd week of gestation or with a birth body weight less under 1500 g. In this group, the incidence of hypothyroxinemia is about 25%. The question is which children who do not meet these criteria (older and heavier) should undergo additional TSH and FT4 serum tests in order not to overlook hypothyroxinemia. After analyzing the cases in our group, we came to the conclusion that they were children with congenital defects of various organs, both the CNS and the gastrointestinal tract, and their condition was severe. Also, Yoon et al. (23) wrote that transient hypothyroxinemia in extremely low birth weight infants is associated with mortality and composite morbidities and the initial T4 level is the most effective for predicting outcome in them. Therefore, it seems that these are children in whom additional TSH and FT4 tests are worth doing after birth.

Many reports emphasize that the hypothyroxinemia observed in premature infants is transient and that the test should be repeated (24–26). However, in our material, in 50% of the children who were not treated after obtaining the first result, hypothyroxinemia was still observed in the subsequent test, performed after a period of 2-5 weeks. This means that during the time which is crucial for the development of the brain, the concentration of FT4 was insufficient.

However, the reports of the benefits of LT4 treatment in premature infants with hypothyroxinemia are divergent (4, 14, 27, 28). In our group, it was difficult to predict in which children hypothyroxinemia would persist and in which it would be transient and FT4 would normalize without treatment. Those two subgroups did not differ in terms of gestational age, birth body weight, or the initial concentrations of FT4, FT3 and TSH. Further follow-up of children with hypothyroxinemia, both those treated with LT4 and those untreated, is being carried out, and an analysis of their condition is planned in a few years time.

Summing up, as hypothyroxinemia affects approximately 25% of newborns born before the 32nd week of gestation and those whose birth weight is less than 1500 g, it seems that in this group of children, the newborn screening should be extended to measure TSH and FT4 concentrations in serum between the 3rd and 5th day of life. Particular attention should be paid to children with SGA and also birth weight lower than average but not meeting the SGA criterion yet), as the prevalence of hypothyroxinemia among them is the highest. It seems that in older and heavier neonates, additional serum TSH and FT4 assessment should be limited to children with severe congenital abnormalities.

Despite the fact that the most common (79.5%) form of preterm hypothyroxinemia is the low FT4 syndrome, i.e. hypothyroxinemia with a normal TSH concentration, it should be emphasized that FT4 remains lowered on subsequent testing in more than 50% of cases.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Bioethical Committee at the Polish Mother's Memorial Hospital-Research Institute (PMMH-RI) in Lodz. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

Conceptualization: MN-B, RS. Methodology: AL, MK-K, TT. Formal analysis: RS, MH, EG. Investigation: RS, MK-K, AL, MN-B, TT. Data curation: EG, MH and AL. Writing original draft preparation: MN-B, TT, RS. Writing review and editing: EG, RS and AL, supervision: AL. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Case report: Goiter and overt hypothyroidism in an iodine-deficient toddler on soy milk and hypoallergenic diet

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Soy-based infant formulas (SFs) are often consumed by cow's milk allergic children. However, some concerns have risen since soy intake may adversely affect thyroid function in iodine-deficient or subclinical hypothyroid individuals. We report the first Italian case of SF induced goiter and hypothyroidism registered in our country since National Iodine program has been instituted. Finally, we review cases previously reported in literature. A 22-month-old toddler with a previous diagnosis of cow's milk protein allergy came to clinical attention for important goiter and overt hypothyroidism. Detailed dietary anamnesis revealed that he was on a restrictive dietary regimen based on soymilk since 12 months of age. A temporary levothyroxine substitution was instituted to avoid hypothyroidism complications. Adequate iodine supplementation and diet diversification completely reversed SF-induced hypothyroidism and goiter, confirming the diagnostic suspicion of soymilk-induced thyroid dysfunction in a iodine-deficient toddler. This case report demonstrates the importance of careful dietary habits investigation and adequate micronutrients supplementation in children on a restrictive diet due to multiple food allergies in order to prevent nutritional deficits.

KEYWORDS

children, goiter, hypothyroidism, soybean, cow's milk allergy, case report

Introduction

Soy-based infant formula (SF) has been massively used as an alternative diet in children with cow's milk allergy or lactose intolerance (1). However, in the early to mid-

1900s, the discovery of a possible thyreotoxic effect of phyto-estrogenic isoflavonoids contained in soybean has risen some concerns about SF use in infants. Isoflavonoids inhibit thyroid peroxidase (TPO) by acting as alternative substrates for iodination (2–4). By contrast, later studies demonstrated that this effect becomes clinically relevant only when iodine intake is insufficient (5, 6). A systematic review in 2007 reported that modern iodine-enriched SFs adequately support growth and development. Indeed, no case of goiter and/or hypothyroidism has been described with iodine-enriched SFs. The combined effect of soy foods consumption and iodine deficiency on thyroid volume and function was later confirmed in rodents models (2, 7, 8).

Here, we reported the case of a 22-month-old toddler who was put on a soy-based diet due to cow's milk protein allergy and developed goiter and overt hypothyroidism. This is the very first case in Italian population and, to the best of our knowledge, a unique report in countries where the Iodine Deficiency Disorders Control Programme is ongoing. We retrospectively analyzed other case reports in humans and synthesized available data on thyroid dysfunction risk associated with SF.

Case presentation

A 22-month-old Italian male infant presented at the outpatient Pediatric Endocrinological service of the A.O.U. "Luigi Vanvitelli" with a 3-month history of progressive anterior neck swelling. All clinical data, in here presented, and related images have been disclosed in accordance with the Helsinki declaration, after both parents gave informed consent to publication and data were anonymized.

Familial history was relevant for autoimmune-thyroiditis-induced hypothyroidism in the mother. Mid-parental height was 177 cm (+0.07 SDS). The baby was the only child of unrelated parents. He was born full-term from an uncomplicated pregnancy. At birth, he weighted 3.660 kg (+1.21 SDS according to Neonatal Anthropometric Charts for the Italian population, Bertino et al. 2010), length was 50 cm (+0.26 SDS), and head circumference was 35 cm (+0.69 SDS). The mother's thyroid function was normal during pregnancy and received no drug affecting thyroid function. The baby passed neonatal screening for congenital hypothyroidism. He had been formula fed since birth. Clinical history revealed normal psico-motor development. At 4 months of age, he suffered from food-protein-induced enterocolitis syndrome; since then, he followed a cow's milk protein-free diet. Hence, the child only received extensively hydrolyzed formula until 1 year of age. Then, he was switched to soy-based infant formula. The weaning process was never completed due to his parents' fear of food allergy reactions. At 22 months of age, the baby diet was very selective: soymilk represented the main nutritional source (with a daily intake of 800–1,000 ml) associated with small amount of carbohydrates (rice or pasta). No protein-rich foods (fish, meat, and eggs) were provided. Moreover, he had a low-salt diet; therefore, iodine intake appeared inadequate.

On physical examination, auxological parameters were within normal according to the WHO growth chart, 2006: he weighed 10.650 kg (−0.88 SDS), his height was 83.5 cm (−0.87 SDS), and his head circumference measured 48 cm (+0.79 SDS). Neck palpation revealed a soft, non-tender, diffuse swelling of the thyroid gland (shown in Figure 1). He showed dry skin, thin hair, and delayed teeth eruption.

Investigation and management

Thyroid ultrasound confirmed thyromegaly: gland volume measured 8.7 ml (> +1 SD according to age and sex reference values) with no underlying mass. Laboratory testing showed elevated serum thyrotropin concentration [thyroid-stimulating hormone (TSH), 47.510 μU/ml; normal range, 0.1–4.1], low serum-free tetraiodothyronin (FT4, 1 pg/ml; reference value, 4.4–12 pg/ml), and normal free triiodothyronine (FT3, 3.7 pg/ml; reference value, 1.0–4.3 pg/ml). Serum thyroglobulin was increased (TG, 701.5 ng/ml; reference, <60 ng/ml). Thyroid antibodies were not detected. Taking into account the importance of proper thyroid function in the first years of life, the baby was placed at levothyroxine replacement therapy (about 1 μg/kg/die).

Differential diagnoses of SF-induced goiter in iodine-deficient toddlers and congenital hypothyroidism were considered. As the baby presented normal neonatal thyroid screening, a normal thyroid volume, and no symptoms of hypothyroidism in the first year of life, congenital hypothyroidism does not seem likely. Thus, there was a strong suspicion of iodine deficiency, as pointed out by dietary anamnesis. Therefore, we first estimated iodine intake, analyzing urinary iodine output from a 24-h urine sample collection. Urinary iodine concentration (UIC) was strongly reduced (<8 μg/L). According to current FAO/WHO daily recommended nutrient intake for iodine, we prescribed a dietary salt intake of 3 g containing about 90 μg of iodine per day (9). Moreover, soymilk was replaced with extensive hydrolyzed formula. Fish, meat, eggs, and fruit were gradually introduced into his diet.

For the sake of completeness, genetic testing was performed in order to rule out congenital hypothyroidism due to thyroid peroxidase (TPO) deficiency. Genomic DNA of our patient was extracted from peripheral whole blood sample, using a DNA extraction kit (Promega, Madison WI, USA). A direct sequencing of *TPO* gene was performed by Sanger method under standard conditions. PCR analysis found no mutation.

The final diagnosis was secondary hypothyroidism due to iodine deficiency, complicated by soymilk consumption.

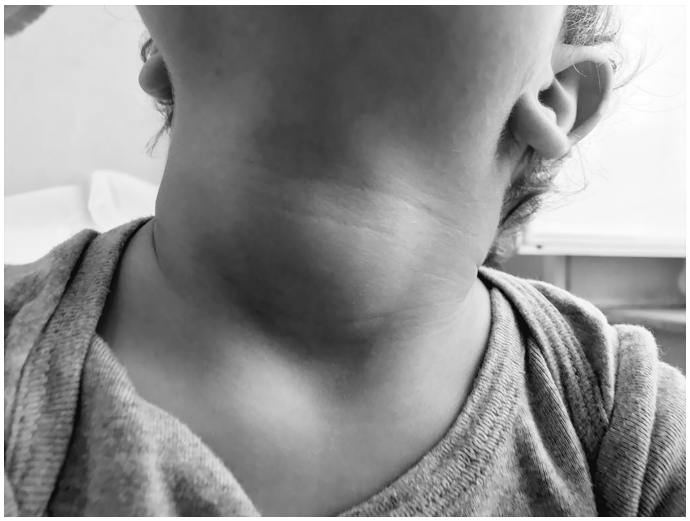

FIGURE 1
Child's neck at diagnosis: thyroid gland was considerably increased in size, soft, non tender and freely movable with swallowing.

Outcomes

Within 3 weeks since the beginning of levothyroxine treatment, thyroid function normalized (TSH, 3.38 mU/L; FT4, 10 ng/ml). Thyroglobulin decreased (138 ng/ml), although not within normal range. Gland size remarkably reduced. After 3 months of supplementation, patient showed increased growth rate, good general conditions, and reduced thyroid volume. Levothyroxine was tapered within 3 months of treatment and stopped at 25 months of age. In the first year of follow-up, thyroglobulin and median UIC completely normalized. At the age of 3.5 years, the baby maintained in the euthyroid state, and thyroid gland was no longer palpable. Auxological parameters were normal [height, 97.7cm: +0.58 SDS; weight, 14 kg; and body mass index (BMI), 16.5 kg/m^2]. At the age of 6.25 years, height was 124 cm, +1.3 SDS; he weighed 21.2, and his BMI was 18.6 kg/m^2. Thyroid function was normal (TSH, 0.937 μU/ml, FT4, 10.2 pg/ml). The growth chart is shown in Figure 2.

Discussion

This case is unique because the timeline of events suggests that severe biochemical hypothyroidism and goiter can be triggered by SF in children on very selective diet. To our knowledge, this is the first case report of soy-induced goiter in countries where an iodization program is ongoing. In Italy, national iodine prophylaxis has been introduced since 2005 with the approval of Law 55/2005, which regulates the sale and use of iodized salt (iodine content, 30 mg/kg of salt). Periodic reports by the National Observatory for the Monitoring of Iodine Prophylaxis in Italy (OSNAMI) of the ISS demonstrated that Italy achieved iodine sufficiency since 2015 (10).

Etiopathogenesis of soy-induced goiter and overt hypothyroidism has been widely investigated in the literature. Soy isoflavonoids (genistein and daidzein) act as goitrogenic substances by the following mechanisms: (a) inhibiting the iodination activity of TPO and (b) increasing hormonal iodine fecal losses. However, animal and human studies data point out that soy-induced overt hypothyroidism and goiter are uncommon in the absence of other risk factors. Ikeda et al. (2) demonstrated, similarly to Kimura et al. (7), that feeding rats a soy-containing diet caused severe hypothyroidism only when iodine deficiency was present. In 2017, a recent systematic review carried out in the general population revealed that euthyroid individuals with adequate iodine intake had modest isolated TSH rise with no significant changes in FT3 and FT4 levels when exposed to soy isoflavonoids (6). By contrast, in the presence of subclinical hypothyroidism or risk factors for thyroid dysfunction, such as iodine deficiency, as in our patient, soy isoflavonoids can produce clinically significant effects.

We performed a review of all cases of soy-induced goiter and/or hypothyroidism recorded in the literature. We search in PubMed database and Google scholar with terms: ["children"AND/OR"infant"] AND ["soybean"OR/AND"soy-milk"OR/AND"soy-based formula"] AND ["goiter"AND/OR"thyroid dysfunction"]. Results focused on case reports published in English. A total of six reported cases was

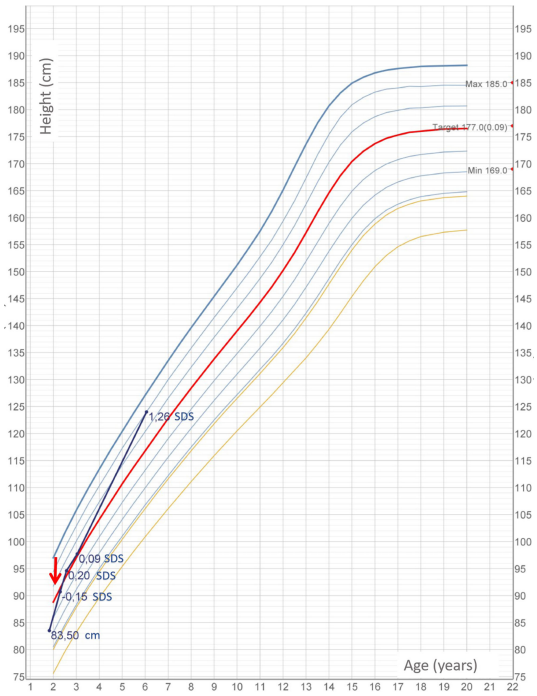

FIGURE 2
Child's height measurements according to Cacciari growth chart 2006 for Italian population. Red arrow marks beginning of euthyroid status.

identified; no case has been reported after 1995. The presentation and management of patients are summarized in Tables 1, 2. Two reports pointed out the risk of interference of SF with levothyroxine substitutive therapy in congenital hypothyroidism (see papers 5 and 6). In these cases, the initial increase in medium therapy dosage partially addressed hypothyroidism, while whole cow's milk substitution totally stabilized the euthyroid state and prevented from further therapy dosage increases. The remaining cases shared in common no signs of previous thyroid impairment before soy intake, familial or personal history of protein allergy, and no clinical sign of hypothyroidism. Intervention for goiter correction varies among cases, from SF stopping to iodine addition to diet.

Our case report partially differs from others previously reported, as our patient developed an overt hypothyroidism and temporarily needed levothyroxine supplementation in addition to long-term dietary modifications. Age of goiter and hypothyroidism onset, 22 months old, was older than previously reported in the literature. Delayed onset, in our patient could be explained by partial iodine substitution within the first year of life due to specific infant formula, which are iodine fortified.

The clinical history of this child supports the previous evidence of synergism between excess in soybean intake and

TABLE 1 Laboratory findings during follow-up. Abbreviations: free Thyroxine(fT4), Thyroid.

Age months	TSHμ UI/ml	FT4 pg/mL	Tg μg/L	UIC μg/L	THERAPY (Levothyroxine)
22 (diagnosis)	47.5	1	701	<8	Start therapy1 μg/kg/die
22	3.38	10	138		1 μg/kg/die
23	1.509	13.2	97.43	70	0.5 μg/kg/die
24	1.85	10	71.24		Stop therapy
27	0.9	13.2	54.17	>100	no therapy
30	0.8	11.4	56.09		no therapy
36	0.8	9.7	45.01		no therapy
42	0.9	11.1	38		no therapy
75	0.94	10.2	31.21		

Stimulating Hormone (TSH), Urinary iodine concentration (UIC) and Thyroglobulin (Tg).

TABLE 2 Literature review of presentation and management of patients.

	Author Year of publication	Journal	Age and gender	Duration of soymilk formula consumption	Clinical examination	Intervention
1 (11)	Hydovitz JD.1960,	N Engl J Med	5 months Male	4 months	Diffuse thyromegalia of rubbery consistency. Normal growth pattern. No clinical signs of hypothyroidism.	Cow's milk substitution with remission in one month.
2 (12)	Shepard TH 1960 United States	N Engl J Med	10 months Female 3 months Female 39 months Female	6 months Since birth 36 months due to eczema experienced at 3 months of age	Goiter, but euthyroid Goiter, but euthyroid Goiter, but euthyroid	Soybean formula discontinuation at 11 months of age with total remission in 3 month and half Cow's milk substitution with remission after 1.5 months. Q[CE] Please provide the exact mountain Lugol's solution with remission after 15 days.
3 (13)	Ripp JW. 1961 United States	Am J Dis Child	15.5 months Male	12 months since he experienced a severe eczema and spitting at 3.5 months.	Diffuse soft swelling of all lobes of the thyroid gland. Normal growth pattern. no clinical signs of hypothyroidism.	Goat's milk was substituted for soybean milk with rapid remission. After, total recovery, at 21.5 months of age, a trial with soy-milk for 80 days, demonstrated to cause slight thyroid volume increase.
4 (14)	Van Wyk JJ 1959 United States	Pediatrics	8 months Female,	Since birth due to a family history of strong allergies until 2 week prior first visit	Diffuse thyromegalia and sign of hypothyroidism (puffy face, marked pallor, thick and protruding tongue, peripheral mottling and carotenoid complexion) Length deficiency NO mental retardation	Lugol's solution and replacement of the soybean product by whole cow's milk. Total remission in 14 months
5 (9)	Pinchera A 1965 United States	N Engl J Med	6 months and 3 weeks Male Congenital hypothyroidism on substitutive treatment since 3 months and half	7 week after milk allergy symptoms appeared at 5 months of age and necessity to increase thyroxine therapy	Athyreotic with partial clinical sign of hypothyroidism (puffy face, dry and mottled skin, pallor, irritability, alert). Reduced lenght growth.	replacement of the soybean product by whole cow's milk with rapid recovery
6 (15)	Chorazy PA et al. 1995 United States	Pediatrics	1 month and 19 days Male Congenital hypothyroidism on substitutive treatment since day 11 of life	Since birth due to family history of cow's milk intolerance	Difficulty in normalizing thyroid tests. Increased stool frequency	Whole-cow milk diet restored and adjustment of levothyroxine therapy dosage for body weight

iodine deficiency to induce goiter and hypothyroidism (2–4). After SF was eliminated and a varied and balanced diet was introduced, euthyroidism was achieved and goiter completely reverted. In addition, genetic analysis performed on *TPO* gene ruled out any possible mutation encoding a partially activated TPO protein that could partially explain or contribute to our child phenotype.

Although a recent review in 2018, investigating global phytoestrogens effects on growing child found soymilk formula are not associated with relevant abnormalities (16), no specific investigation has been carried out on the effects of restrictive soymilk diet on thyroid function in otherwise healthy children. That limits the indications of SF use in infant nutrition. According to the American Academy of Pediatrics (17) and the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (18), SF has a specific indication for full-term infants suffering from galactosemia and hereditary lactase deficiency. In case of cow's milk protein allergies, as in our case, different nutritional approaches are preferred. However, it is not unusual in clinical practice to use SF in multiallergic patients as ESPGHAN recent guidelines for cow's milk protein allergy identify soymilk as a possible second option in babies younger than 6 months who do not tolerate extensively hydrolyzed infant formulas (19).

That case highlights the importance of regularly assessing micro-nutrients in children by visiting a registered dietitian following elimination diets due to multiple food allergies. A detailed nutritional anamnesis can help specialist to detect

patients at risk of iodine deficiency. Unfortunately, no specific diagnostic tool has been validated to assess individual iodine intake. In the case herein presented, assessment of UIC on 24-h urinary collection has been performed to gain some information on the iodine intake for the past few days. We acknowledged that UIC cutoffs have been developed to assess population iodine intake and is not recommended as a diagnostic tool for iodine deficiency in a given individual, as high intra-individual day-to-day variability exists (20). However, considering that UIC reflects iodine intake shortly before sampling and variability is due to different natural iodine content and bio-availability of food items, mean iodine levels are presumably constant over days in the case of very restrictive eating habits. Thus, we can reasonably consider that a UIC <100 µg/L in our child pointed out iodine deficiency status. Moreover, circulating thyroglobulin levels were increased in accordance to the correlation existing on a population level between this specific thyroid marker and iodine deficiency (16). In the case herein presented, the high thyroglobulin values and the low levels of UIC reflect the deep and protracted iodine deficiency due to the lack of adequate complementary nutrition and worsened by large consumption of soy-based formula.

Iodine fortification of allergy formulas may be insufficient in some cases, and the risk of iodine deficiency with selective diets regimens increases over time, so an additional iodine supplementation should be tailored on a single patient.

Conclusions

Despite significant improvements in iodine status of populations worldwide, iodine deficiency continues to be a possible cause of goiter and/or hypothyroidism, especially in multiple food allergic infants on restrictive diet. Particular attention should be paid to the eating habits of these children in order to prevent dangerous nutritional deficits, including iodine deficiency. In addition, some foods can have a negative effect on thyroid function. In conclusion all children, especially children fed by soy formula, should receive complementary feeding with an adequate iodine content, in order to maintain a regular thyroid function.

Data availability statement

The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding author.

Ethics statement

This study was reviewed and approved by University of Campania Luigi Vanvitelli. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

AC and GU prepared the original draft manuscript. FA and GU review and edited the manuscript. CL managed the hypothyroidism. II was responsible of food allergy management. SP performed genetic testing. EG and AG supervised the writing of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the present case report was published in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Effect of initial levothyroxine dose on neurodevelopmental and growth outcomes in children with congenital hypothyroidism

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Objectives: We designed a multicentre open prospective randomized trial to evaluate the risk-benefit profile of two different initial treatment schemes with levothyroxine (L-T4), 10-12.5 µg/kg/day vs 12.6-15 µg/kg/day, on growth and neurodevelopmental outcomes in children with congenital hypothyroidism (CH) detected by neonatal screening to identify the best range dose to achieve optimal neurocognitive development.

Design, patients and methods: Children detected by neonatal screening were randomly assigned to receive an initial L-T4 dose of 10-12.5 µg/kg/day (Low) or 12.6-15 µg/kg/day (High). All patients underwent periodical clinical examination with measurement of growth parameters and measurement of TSH and FT4. Neurocognitive development was evaluated at the age of 24 months using Griffiths Mental Development Scales (GMDS) and cognitive and behavioral assessment was performed at 48 months of age using Wechsler Preschool and Primary scale of Intelligence (WIPPSI-III). The study was registered with clinicaltrials.gov (NCT05371262).

Results: Treatment schemes below or above 12.5 µg/kg/day were both associated with rapid normalization of TSH and thyroid hormone levels in most patients with no differences in the risk of over- and under-treatment episodes in the first months of life. Growth parameters were normal and comparable between the two groups.

Developmental quotients at 24 months of age were normal in both groups (Low 100.6 ± 15.5 vs High 96.9 ± 16.6). Likewise, at 4 years of age IQ and subtest scores were comparable between patients from Low and High (Total IQ 104.2 ± 11.4 vs 101.0 ± 20.3, Verbal IQ 103.9 ± 11.5 vs 98.7 ± 15.1, Performance IQ 105.3 ± 10.4 vs 100.3 ± 19.8). 6/45 CH patients (13.3%) showed a total IQ below 85 (73.7 ± 5.9) regardless of age at diagnosis, L-T4 starting dose, time of FT4 and TSH normalization and episodes of over and undertreatment. Worse socioeconomic status and delayed bone age at diagnosis were the only predictors of an increased risk of having suboptimal IQ at 24 and IQ at 48 months.

Conclusions: Our results indicate that initial treatment with L-T4, 10-12.5 µg/kg/day vs 12.6-15 µg/kg/day, are both associated with normal growth and neurodevelopmental outcomes in children with CH detected by neonatal screening. Further studies with a long-term follow-up on a larger number of patients are needed to confirm these results.

Clinical trial registration: https://clinicaltrials.gov/ct2/show/NCT05371262?term=NCT05371262&draw=2&rank=1 identifer NCT05371262.

KEYWORDS

neonatal screening, congenital hypothyroidism, levothyroxine treatment, neurocognitive, growth

Introduction

Thyroid hormones are essential for nervous system development as they regulate several brain processes such as neuronal proliferation and migration, growth of axons and dendrites, myelination and synaptogenesis (1–3).

Congenital hypothyroidism (CH) is considered one of the most common preventable causes of intellectual disability (4).

Screening programs have led to early detection and treatment of infants with CH thus preventing the severe neurocognitive impairment resulting from late diagnosis (5) and allowing the achievement of normal adult height (6, 7).

Short- and long-term studies on neurocognitive function in early treated patients with CH have shown that, despite optimal cognitive development is achieved in the majority of CH patients, subtle neurocognitive deficits may still occur (8, 9).

Guidelines for CH recommend starting treatment with a dose of levothyroxine (L-T4) between 10 and 15 µg/kg/day, which has been associated with a faster normalization of both TSH and FT4 levels (10, 11). A recent systematic review suggests that only a starting dose >10 µg/kg/day is able to guarantee a normal neurocognitive outcome in patients with both severe and moderate CH (12). However, concern has been raised on the negative long-term effects of high initial doses of L-T4 on behavior and neurocognitive development due to the increased risk of overtreatment (13–17).

So far, only one randomized controlled study compared the effects of different L-T4 initial doses within this recommended range (18). Despite a few methodological limitations (19) the results of this study suggest that the highest is the initial dose in the treatment of CH the earliest is the achievement of euthyroid status and the best is the intellectual outcome (18, 20).

We designed this multicentre open prospective randomized trial to evaluate the risk-benefit profile of two different initial treatment schemes with L-T4, 10-12.5 µg/kg/day vs 12.6-15 µg/kg/day, on growth and neurodevelopmental outcomes in children with CH detected by neonatal screening to identify the best range dose to achieve optimal neurocognitive development.

Furthermore, secondary objective of this study was to evaluate the role of factors other than dose potentially influencing long-term growth and neurodevelopmental outcomes in children with CH.

Materials and methods

Study protocol

Six Italian centers were involved in this multicentre open prospective randomized trial. Patients were enrolled from May 2011 to May 2014. The study was registered with clinicaltrials.gov (NCT05371262).

In all subject recalled by neonatal screening, diagnosis of CH was confirmed by measurement of venous TSH, FT4 and FT3 together with measurement of thyroglobulin and thyroid autoantibodies.

Inclusion and exclusion criteria were defined in order to reduce the risk of enrolling subjects carrying factors potentially affecting growth and neurodevelopmental outcome, moreover a TSH cut-off greater than 30 mU/l was arbitrarily chosen to select patients who necessarily required treatment. Inclusion criteria were: age at diagnosis less than 30 days, Caucasian ethnicity and TSH at diagnosis above 30 mU/l. Exclusion criteria were: prematurity, major malformations, neonatal diseases, cromosomopathies and maternal thyroid disease.

Children with CH who fulfill both inclusion and exclusion criteria were enrolled and randomly assigned to receive an initial L-T4 dose of 10-12.5 µg/kg/day (Low) or 12.6-15 µg/kg/day (High). Randomization was designed according to a block scheme (8 blocks of 6 patients and 6 blocks of 4 patients which were randomly alternated) which guaranteed the frequency balance in the two groups during the enrolment without altering the causality of the assignment. Random allocation sequence was generated using the function *sample* in R statistical platform.

Bone maturation was assessed in all CH subjects at diagnosis, by evaluating the presence and the diameter of the epiphyseal nucleus of distal femoral at knee X-rays. Neonatal bone maturation, which is considered an indicator of intrauterine and severe CH, was considered delayed when the distal femur bony nucleus diameter was below 3 mm (21).

Thyroid morphology was assessed by thyroid ultrasound at diagnosis and by thyroid scan with either iodine-123 or technectium-99 at diagnosis or at the age of 36 months after L-T4 withdrawal.

Patients with eutopic gland, underwent diagnostic re-evaluation, at 3 years of age, after therapy withdrawal, to identify subjects with permanent vs transient CH.

Clinical and biochemical evaluation

All patients underwent clinical examination and measurement of TSH and FT4 levels 7-10 days after the start of treatment and at 1.5, 3, 6, 9, 12, 18, 24, 30, 36, 42 and 48 months of life. Clinical evaluations included measurement of growth parameters (weight, length, cranial circumference) and the assessment of signs and symptoms of under- or over-treatment. Weight and length were expressed as standard deviation score (SDS) (22).

Initial L-T4 dose was modified, when necessary, in order to maintain serum TSH between 0.5 and 4.0 mU/l and serum FT4 in the upper normal range for age (1.4-2.3 ng/dl) (10). To evaluate the effects of under- or over-treatment during the follow-up on neurodevelopmental outcomes, we arbitrarily defined as index of under-treatment the number of episodes when serum TSH was >4.0 mIU/l and/or FT4 <1.4 ng/dl, and of over-treatment the number of episodes when serum FT4 was >2.3 ng/dl and/or TSH <0.4 mIU/l.

TSH and FT4 serum concentrations were measured by electrochemiluminescence immunoassay using a commercial kit (Roche Diagnostics) (reference ranges, TSH, 0.3-4.2 mIU/L; FT4, 0.75-1.7 ng/dL).

Intellectual evaluation and socioeconomic status

Neurocognitive development in CH patients was evaluated at the age of 24 months using Griffiths Mental Development Scales (GMDS). The GMDS provides an overall developmental quotient (DQ) with subscales assessing skill areas: locomotor (subscale A), personal-social (subscale B), hearing and speech (subscale C), eye-hand coordination (subscale D) and performance (subscale E).

At 48 months of age cognitive and behavioral assessment was performed using Wechsler Preschool and Primary scale of Intelligence (WIPPSI-III). The WIPPSI-III evaluates the intelligence of children between 2.6 and 7.3 years and provides a Total Intelligence Quotient (TIQ), a Verbal Intelligence Quotient (VIQ), a Performance Intelligence Quotient (PIQ) and a Processing Speed Quotient (PSQ) for children from 4 years of life. WIPPSI-III for 4 years old children consist of 8 main subtests: Information, Vocabulary and Word Reasoning for VIQ; Block Design, Matrix Reasoning and Picture Concepts for PIQ; Symbol Search and Coding for PSQ.

All psychologists from different centers performed a training course in order to ensure homogeneous neurodevelopmental evaluations; moreover, they were blinded about the group the subjects belonged to.

The socioeconomic status was evaluated using the revised Graffar score (23), which distinguishes 5 socioeconomic levels according to the education and occupation of each parent, the main source of income of the family, and the home's condition. A higher score indicates a lower socioeconomic status.

Informed consent was obtained from all parents and the study was approved by the Ethics Committee of University Hospital Federico II of Naples (protocol number 186/09).

Outcomes

The primary outcome was the WIPPSI-III score for the Total Intelligence Quotient at 48 months. Secondary outcomes were the WIPPSI-III scores for Verbal Intelligence, Performance Intelligence and Processing Speed Quotients at 48 months and the GMDS, both DQ and subscales A, B, C, D and E at 24 months.

Sample size

Sample size was estimated based on a clinically relevant between-groups difference in the WIPPSI-III Total Intelligence Quotient at 48

months equal to 5 points. Assuming a common standard deviation in the two populations equal to 5, a sample size of 27 children per treatment arms was deemed sufficient to detect such difference, if truly exists, with a two-sided significance level of 0.05 and a power of 0.80. Considering a drop-out rate of approximatively 20% and taking into account that about 5% of patients will have a transient form of CH and therefore will be excluded from the study, 72 children, 36 per treatment arm, was enrolled in order to have complete data for the primary end point analysis.

Statistical analysis

We analyzed the primary endpoint and all secondary endpoints using the modified intention-to-treat (ITT) population defined as all randomized patients who received at least one dose of study treatment and for whom outcome data were available. For the cognitive endpoints, a sensitivity analysis was conducted in which missing outcome data were imputed using a multiple imputation approach. Variables entered in the imputation model were, besides treatment arm, age at diagnosis, Graffar score, severity of hypothyroidism, bone maturation and parameters of thyroid function at diagnosis (TSH and FT4). As the results of Multiple Imputation were practically equal to the complete-case analysis, only these were reported in the paper.

Demographic and clinical data referred to the baseline were summarized using standard descriptive statistics and compared between group (without reporting statistical significance) to assess whether good balance was achieved by randomization.

Primary and secondary end points were compared between groups using t-test for independent samples. Magnitude of effect was reported as mean difference with the corresponding 95% Confidence Intervals (95% CIs).

Longitudinal trajectories of thyroid function (TSH and FT4) and growth parameters (weight and height) during the follow-up period, were analyzed by using random-intercept linear mixed model (LMM) in which time from baseline was treated as categorical factor. Results of LMMs were reported as Estimated Marginal Means (EEMs) with the corresponding 95% CIs.

The exploratory analysis of potential predictors of long-term neurodevelopmental outcomes was based on univariable linear and logistic regression models, according to the numerical or dichotomic nature of the outcome variable. Results of these models are reported as mean differences and odds ratios (OR) with the corresponding 95% CIs.

All statistical analyses were conducted using the statistical platform R. *mice* package was used for multiple imputation.

Results

One hundred twenty-five CH patients were assessed for enrolment in the study, 53 were excluded because did not fulfil all inclusion criteria or because parents refused to participate in the study. Thus, 72 patients were enrolled in the study and randomised in the two treatment groups.

During the follow up period, twenty-seven (37.5%) patients dropped out from the study because of poor attendance at protocol schedule. The modified ITT population thus consisted of 45 patients followed longitudinally in the first 4 years of life (Figure 1).

Baseline characteristics of the patients divided in the two treatment groups 10-12.5 µg/kg/day vs 12.6-15 µg/kg/day at study entry are reported in Table 1. Overall, mean age at diagnosis was 13.53 ± 6.20 days. Severe hypothyroidism, defined as FT4 concentrations at diagnosis <0.4 ng/dl was observed in 34.8% of patients, with no differences between groups (36.4 vs 33.3%). The remaining 65.2% had moderate CH. Delayed bone maturation at the knee radiography was detected in 28% of patients. Thyroid scan revealed eutopic thyroid in 38.8% of patients and dysgenesis in the remaining 61.2%; in the latter group retrolingual ectopy was found in 40.3% and thyroid agenesis in 20.9% of patients. Overall, the two treatment groups were balanced, the only difference between the two groups was the L-T4 dose at baseline as expected based on the study design (Table 1).

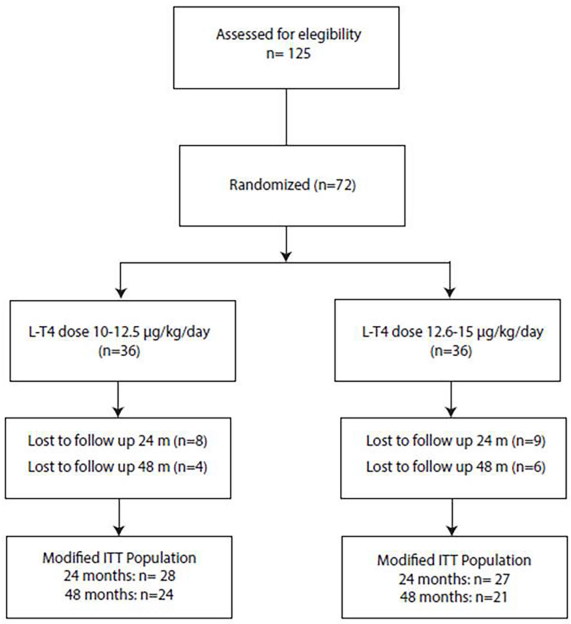

FIGURE 1
Flow-chart of the recruitment process. Of the 125 potentially recruitable patients with CH 53 were excluded because did not fulfil all inclusion and exclusion criteria or because parents refused to participate in the study. Thus, 72 patients were enrolled in the study and randomised in the two treatment groups. During the follow up period, 17 patients drop out from the study before 24 months of life and additional 10 were lost to follow up after 24 months. Thus, the modified ITT population consisted of 45 patients followed longitudinally in the first 4 years of life.

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TABLE 1 Baseline characteristics of patients randomized in the two groups of treatment at the enrollment in the study.

	Low (10-12.5 µg/kg/day)	High (12.6-15 µg/kg/day)
Number of patients	36	36
Sex Female/Male (%)	61.8/38.2	57.6/42.4
Gestational age (weeks)	39 (37-41)	40 (37-41)
Graffar Score	13 (4-18)	14 (4-18)
Age at diagnosis (days)	13.36 ± 5.55	13.71 ± 6.85
Moderate CH/Severe CH (%)	63.6/36.4	66.7/33.3
Adequate bone maturation/Retarded bone maturation (%)	80/20	64/36
Eutopic gland (%)	46.2	25
Ectopy gland (%)	38.5	45.8
Athyreosis (%)	15.4	29.2
TSH at diagnosis (mIU/l)	296.0 ± 235.0	341.2 ± 279.7
FT4 at diagnosis (ng/dl)	0.55 ± 0.31	0.53 ± 0.34
Initial L-T4 dose (µg/kg/day)	11.69 ± 0.65	13.47 ± 0.84

Data are expressed as percentage, median and range or mean ± standard deviation.

Thyroid function

According to study protocol, patients were evaluated 7-10 days after starting L-T4 treatment (corresponding to a mean chronological age of 22.70 ± 6.25 days) and subsequently at 1.5 months of age (less than 1 month after the first post treatment evaluation).

Serum concentrations of TSH and FT4 were comparable in the two groups throughout the follow-up (Figures 2A, B). L-T4 dose was higher in High at baseline and remained significantly

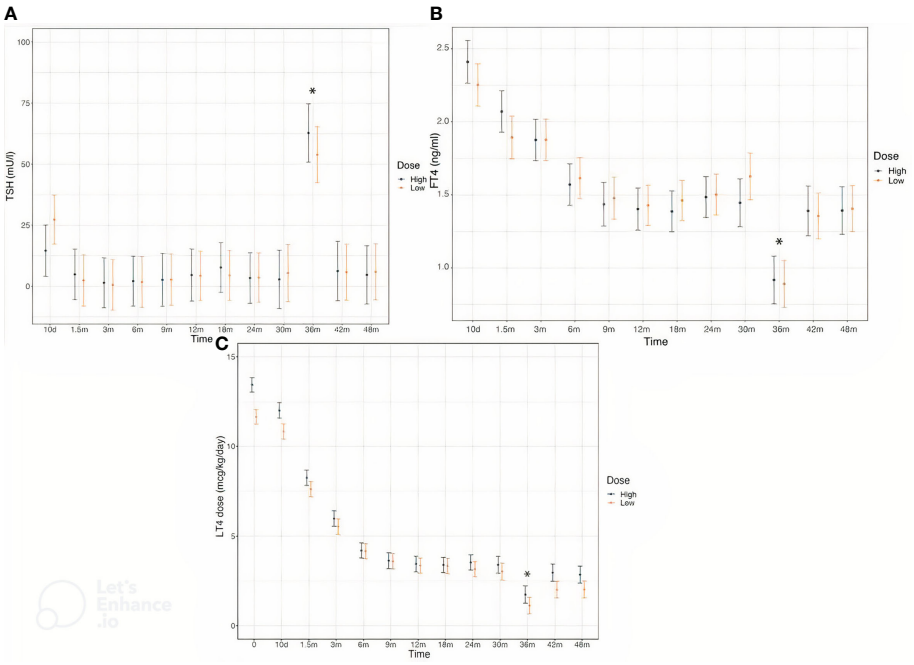

FIGURE 2
Serum concentrations of TSH **(A)**, FT4 **(B)** and L-T4 dose **(C)** in CH patients divided in the two treatment groups throughout the study. According to study protocol, thyroid hormones levels were evaluated at the enrollment, 10 days after L-T4 start and subsequently at the chronological age of 1.5, 3, 6, 9, 12, 18, 24, 30, 36, 42 and 48 months of life. * Changes in TSH and FT4 levels and in L-T4 dose at the age of 3 years are due to L-T4 withdrawal in the majority of patients for the re-evaluation thyroid function.

different in the first 7-10 days after therapy initiation, by the age of 1.5 months this difference was no longer appreciable (Figure 2C). The frequency of dose adjustment during the first six months of treatment as well as in the subsequent months was similar in both groups.

No differences in the number of episodes of over- and under-treatment during the first 6 months of therapy were recorded in the two groups of patients.

Growth

Weight and length/height were normal and comparable between the two treatments groups all over the study period as depicted in Figure 3.

Neurocognitive development

All patients were euthyroid at the time of neurocognitive evaluations. Overall, at 24 months of age mean developmental quotients (DQ 98.80 ± 15.98) and subscale scores were normal (Subscale A 110.64 ± 22.22, Subscale B 96.98 ± 23.17, Subscale C 89.18 ± 20.57, Subscale D 103.05 ± 15.66, Subscale E 103.71 ± 15.02). No significant differences were observed when CH patients were divided on the basis of the initial treatment regimen both in the modified ITT population (Table 2).

Similarly, at 4 years of age CH patients had normal Intelligence Quotient scores (TIQ 102.71 ± 16.02, VIQ 101.47 ± 13.41, PIQ 103.00 ± 15.51, PSQ 98.24 ± 15.59). IQ and subtest scores at 48 months of age were comparable between patients from Low and High (Table 2).

Six out of 45 CH patients (13.3%) showed a total IQ below 85 (73.7 ± 5.9) and of them only 1 had an IQ below 70 (2%). In details, mean QIV was 79.7 ± 7.5, mean QIP was 73.2 ± 6.7, mean PSQ was 74.3 ± 5.5. Five out of 6 patients were treated with a L-T4 initial dose above 12.6 µg/kg/day, 3/6 had athyreosis with severe CH and delayed bone age at diagnosis, 1/6 had ectopic and 2/6 eutopic gland and all had high Graffar score indicating a low socioeconomic status. 5/6 patients experienced 2-4 episodes of over-treatment while the remaining 1 experienced 3 episodes of under-treatment within the first year of treatment.

Table 3 summarizes the impact of socioeconomic status and bone age retardation on neurocognitive outcome. A unit increase in Graffar score (which means a worsening of socioeconomic status) was associated with an increased risk of having suboptimal DQ at 24 and IQ at 48 months. Conversely, a unit increase in the diameter of the distal femoral epiphyseal nucleus (which means a less severe CH) was associated with a reduced risk of suboptimal DQ and IQ.

The presence of at least one episode of under-treatment in the first six months of life was associated with higher risk of impaired locomotor outcome at 24 months of live (OR: 5.45, 95% CI: 1.16 to 30.25, p=0.036); the higher the number of the episodes the lower was locomotor quotient at 24 months (mean difference for each incremental episode: -7.8, 95% CI: -15.3 to -0.3, p=0.041).

Conversely, the presence of at least one episode of FT4 levels above range in the first six months of life were associated with reduced risk of impaired verbal outcome at 48 months of life (OR: 0.18, 95% CI: 0.02 to 0.91, p=0.042) and the higher the number of over-treatment episodes the higher was VIQ at 48 months (mean difference for each incremental episode: +8, 95% CI: 2.3-13.6, p=0.007). Moreover, after six months of age episodes of over- or under- treatment were not correlated with neurocognitive outcomes

Overall, the number of over/under-treatment episodes were independent of the L-T4 treatment group the belonged to. In particular, episodes of overtreatment at 12 months were 2.1 ± 1.4

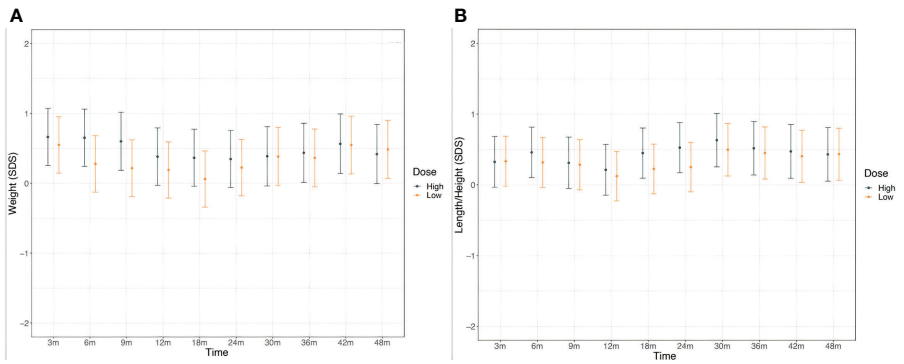

FIGURE 3
Weight **(A)** and height **(B)** in CH patients in the two treatment groups throughout the study. According to study protocol, enrolled patients underwent clinical evaluation at the enrollment, 10 days after L-T4 start and subsequently at the chronological age of 1.5, 3, 6, 9, 12, 18, 24, 30, 36, 42 and 48 months of life.

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TABLE 2 Developmental Quotients and subscale scores at 24 months of age and Intelligence Quotients and subtest scores at 48 months of age in the two groups of patients with different initial treatment regimen.

	Low (10-12.5 µg/kg/day)	High (12.6-15 µg/kg/day)	Between-Group Difference (95% CI)	P
Age 24 months				
Number of patients	28	27		
Developmental quotient	100.6 ± 15.5	96.9 ± 16.6	3.8 (-4.9 to 12.4)	0.39
Subscale A	112.1 ± 21.4	109.1 ± 23.3	3 (-9.1 to 15.1)	0.62
Subscale B	100.5 ± 20.7	93.3 ± 25.3	7.2 (-5.3 to 19.7)	0.26
Subscale C	90.6 ± 19.7	87.7 ± 21.7	2.8 (-8.4 to 14)	0.61
Subscale D	105.4 ± 13.5	100.7 ± 17.5	4.7 (-3.8 to 13.1)	0.27
Subscale E	105.0 ± 14.9	102.4 ± 15.4	2.6 (-5.6 to 10.7)	0.53
Age 48 months				
Number	24	21		
Total Intelligence Quotient	104.2 ± 11.4	101.0 ± 20.3	3.1 (-6.6 to 12.8)	0.54
Verbal Intelligence Quotient	103.9 ± 11.5	98.7 ± 15.1	5.2 (-2.8 to 13.3)	0.20
Performance Intelligence Quotient	105.3 ± 10.4	100.3 ± 19.8	5 (-4.3 to 14.3)	0.31
Processing Speed Quotient	99.2 ± 13.1	97.1 ± 18.3	2.1 (-7.4 to 11.5)	0.67
Information	10.2 ± 2.3	9.1 ± 3.3	1.1 (-0.6 to 2.8)	0.21
Vocabulary	11.5 ± 2.4	10.2 ± 2.6	1.3 (-0.3 to 2.8)	0.10
Word Reasoning	10.3 ± 3.1	10.1 ± 2.8	0.2 (-1.6 to 2)	0.82
Block Design	9.7 ± 2.2	8.6 ± 3.6	1.1 (-0.6 to 2.9)	0.22
Matrix Reasoning	10.7 ± 2.6	11.6 ± 3.6	-0.9 (-2.8 to 1)	0.35
Picture Concepts	11.5 ± 2.8	10.0 ± 3.2	1.5 (-0.2 to 3.2)	0.10
Symbol Search	10.6 ± 2.6	9.5 ± 3.4	1.1 (-0.7 to 3)	0.22
Coding	9.1 ± 2.5	9.6 ± 3.7	-0.5 (-2.4 to 1.4)	0.60

Data are expressed as mean ± standard deviation.

vs 1.8 ± 1.6 (p=0.467) and episodes of undertreatment were 1.1 ± 1.3 vs 0.8 ± 1.1 (p=0.307), in Low vs High respectively.

Multivariable linear and logistic regression models did not reveal significant correlations between severity or etiology of CH and neurocognitive outcomes.

Discussion

The improvement of neonatal screening programs and early treatment with a high initial L-T4 above 10 µg/kg per day has resulted in normal neurodevelopmental outcomes in children

TABLE 3 Impact of Graffar score and femoral nucleus diameter on long-term neurodevelopmental outcomes.

	Graffar score	Femoral nucleus diameter
24 months		
Developmental quotient	**1.39 (1.07 to 2.02) p=0.04**	0.8 (0.56 to 1.12) p=0.2
Subscale A	1.1 (0.89 to 1.42) p=0.43	**0.62 (0.39 to 0.91) p=0.02**
Subscale B	**1.34 (1.1 to 1.73) p=0.01**	**0.71 (0.52 to 0.94) p=0.02**
Subscale C	1.05 (0.91 to 1.21) p=0.54	0.98 (0.77 to 1.26) p=0.89
Subscale D	**1.57 (1.11 to 2.64) p=0.04**	0.75 (0.49 to 1.1) p=0.16
Subscale E	1.18 (0.94 to 1.61) p=0.2	1.06 (0.72 to 1.62) p=0.77
48 months		
Total Intelligence Quotient	**1.51 (1.07 to 2.54) p=0.05**	**0.62 (0.38 to 0.92) p=0.03**
Verbal Intelligence Quotient	1.26 (0.99 to 1.74) p=0.1	0.8 (0.54 to 1.13) p=0.21
Performance Intelligence Quotient	**1.51 (1.07 to 2.54) p=0.05**	**0.62 (0.38 to 0.92) p=0.03**
Processing Speed Quotient	**1.72 (1.19 to 2.95) p=0.02**	**0.66 (0.43 to 0.93) p=0.03**

Data are expressed as Odds Ratio for having a long-term neurodevelopmental outcome lower than clinical threshold (<85 point) for every unit increase in Graffar score or femoral nucleus. Results were obtained using univariable logistic regression models.
Bold values has been used to underline the results but it is not necessary.

and young adults with CH (4) even compared with sibling controls (12, 24).

However, concern has been raised on the negative long-term effects of high initial doses of L-T4 on behavior and neurocognitive development due to the increased risk of overtreatment (13–17).

The results of this multicenter randomized study indicate that different L-T4 starting regimens, within the range of 10-15 µg/kg/day, have comparable effects on growth and neurocognitive outcomes during the first four years of life in children with CH detected by neonatal screening irrespectively from the severity of hypothyroidism. Indeed, treatment schemes below or above 12.5 µg/kg/day were both associated with rapid normalization of TSH and thyroid hormone levels in most patients with no differences in the risk of over- and under-treatment episodes in the first months of life.

In the study by Albert et al. patients were treated from a mean age of nine days with a L-T4 starting dose between 10-15 µg/kg depending on CH severity. No gap was observed comparing 44 CH patients and 53 unaffected sibling controls (24).

In another study from Aleksander et al. the neurocognitive outcome of 76 young adults with CH with a mean age of 18.1 years was compared with 40 sibling controls with a mean age of 19.8 years (12). The median age at diagnosis was eight days and the mean L-T4 starting dose was 13.5 µg/kg per day. There was no difference in overall IQ nor differences in attention, memory, fine motor skills, quality of life scores.

In both studies TSH normalized within a median time of 15 days after diagnosis.

Moreover, in a meta-analysis included in the latter study comparing IQ differences between severe and mild CH cases with respect to the starting dose revealed that even children with severe CH can reach a normal IQ if L-T4 treatment is started with a dose of at least 10 µg/kg (12).

However, a few patients with severe CH may still have subtle cognitive and motor deficits, and lower educational attainment despite early treatment with a high starting L-T4 dose (25, 26).

Recently, Perri et al. evaluated 28 children with permanent CH at a mean age of 9 years. Mean IQ was normal and comparable to controls however, there was a great variability in IQ values with a high percentage of CH patients having sub-optimal IQ (28.6%) and intellectual disability (10.7%). A significant impairment was detected in specific neurocognitive outcomes such as processing speed, visual attention, reading skills and arithmetic which correlated with white matter structure abnormalities (9). In this study the age at the beginning of treatment was quite variable (15.3 ± 7.9 days) as well as L-T4 starting dose (9.46 ± 2.28 µg/kg/day) and the mean TSH values (6.98 ± 4.68 µU/L) at the time of cognitive assessment was mildly elevated in some CH patients.

In our study, all patients were euthyroid at the time of neurocognitive evaluation and mean values of both developmental quotient at 24 months and intelligence quotient at 48 months of age were normal with no differences in specific skills. The percentage of patients with subnormal IQ (13.3%) was quite lower and was independent from age at diagnosis, L-T4 treatment dose and the time required to normalize thyroid function. However, the finding of CH patients with subnormal IQ suggest that neurodevelopmental rescue should not be taken for granted even in the era of neonatal screening (27). Prenatal brain damage due to thyroid hormone insufficiency *in utero*, may not be completely prevented by trans-placental supply of maternal thyroxine and may not be completely reverted by postnatal treatment (9, 28).

On the other hand, overtreatment can also affect neurodevelopmental outcome.

High-dose treatment has been associated with an increased risk of episodes of overtreatment in the first months postnatally, a critical period for brain development, with adverse cognitive outcome in some children with CH (14, 29, 30). In addition, a recent study, in a limited number of CH patients evaluated at 6 and 11 years of age, suggested that over-treatment in the first 1-3 months of life might be associated with attention deficit hyperactivity syndrome whereas under-treatment in the first 3-6 months with behavioral problems indicative for autism (16).

In our study, the number of over and under-treatment episodes in the first 6 months of treatment was independent of the L-T4 treatment regimen and was not associated with adverse neurocognitive outcome at 4 years of age.

To date only one randomized controlled study compared the effects of different L-T4 initial doses within the range of 10-15 µg/kg/day on neurocognitive development reporting higher intelligent quotient in CH children treated with a mean initial dose of 14.5 µg/kg/day compared with children treated with a mean initial dose of 10.9 µg/kg/day (20). However, this study had several limitations such as an unclear randomization and treatment scheme, small sample size and great variability of the age at neurocognitive evaluation.

The strength of our study is that it has been well designed in terms of randomization and that included a large population of children ensuring appropriate evaluation of any differences depending on different doses of L-T4. Moreover, all CH patients received the same protocol of neurocognitive evaluation at the same age. We acknowledge that a major limitation of our study is a sample size of the modified ITT population slightly smaller than that anticipated in the power analysis and a variability of the main outcome measure larger than expected. These limitations could have determined a loss of power that could partly account for the non-significant results. Moreover, another limitation is the short duration of the follow-up, indeed subtle deficits in specific cognitive domains may become detectable at older ages. Finally, thyroid agenesis and ectopic CH may affect the results of the analysis when analyzed together with eutopic CH, as higher doses of L-T4 are generally required in patients with thyroid agenesis and ectopic CH and these disease groups may differ from eutopic CH in the level of hypothyroidism in the fetal period. Actually, the randomized

design of the study, did not allow a separated analysis of data based on different etiologies.

The present study highlights that worse socioeconomic status and delayed bone age at diagnosis were the only predictors of an increased risk of having suboptimal IQ at 24 and IQ at 48 months. In agreement with our findings, other studies have documented a close association between higher social class and either better IQ or better academic achievement (25, 31–34).

Socioeconomic status is an important predictor of neurocognitive performance (35). Indeed, children with lower socioeconomic status are more likely to have worse cognitive abilities (36) and to obtain lower scores on standardized tests of academic achievement (37). Furthermore, a large Korean population-based cohort study reported a significant combined effect for low family income and neonatal hypothyroidism on the risk of intellectual disability in children (38).

Bone maturation at birth has been proposed as marker of prenatal hypothyroidism severity and has been associated with slight neurocognitive deficits (21, 26, 39).

Finally, early detection and treatment of CH are also important to linear growth, onset and progression of puberty, and final height attainment of CH patients (6, 7, 40). In this study all patients showed normal weight and length/height growth regardless of the initial L-T4 starting dose.

In conclusion our results indicate that initial treatment schemes with L-T4, 10-12.5 µg/kg/day vs 12.6-15 µg/kg/day, are both associated with normal growth and neurodevelopmental outcomes in children with CH detected by neonatal screening; however, further studies with a long-term follow-up on a larger number of patients are needed to confirm these results.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, after justified request.

Ethics statement

The study was reviewed and approved by Ethics Committee of University Hospital Federico II of Naples. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

AE has contributed in following the patients, collecting the data, drafting, and revising the manuscript. MCV, MGW, AC, AM, RG, GV, EP have participated to the multicenter study by enrolling and taking care of their patients as well as by revising the manuscript. RDM and DC take care of the patients and revised the manuscript. MP, CP, CB take care of the neurocognitive outcome of the patients and revised the manuscript. DB designed the randomization of the study and performed statistical analysis. MS designed the study, supervised the patients and actively participated in data analysis, drafting and revision of the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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