Neonatal TSH as a marker of iodine nutrition status. Effect of maternal ioduria and thyroid function on neonatal TSH

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Abstract
Introduction: Neonatal thyroid stimulating hormone (nTSH) is a marker of iodine nutrition status in the population. The WHO considers a prevalence of less than 3% of nTSH levels greater than 5 mIU/L in samples obtained within 72h from birth indicative of iodine sufficiency. The aim of this study was to determine the prevalence of nTSH levels greater than 5 mIU/L in an iodine-sufficient population and its association with maternal, neonatal and obstetric factors. Materials and methods: A total of 243 pregnant women were recruited between May and June 2017 in our health area. A questionnaire of iodine intake was administered, in addition to determination of ioduria, thyroid function and autoimmunity in the first trimester of gestation. We analysed nTSH levels in samples collected between 48 and 72h post birth and other obstetric and neonatal factors. Results: The mean nTSH level (standard deviation) was 2.43 (1.68 mIU/L), with 7.8% of neonates having levels greater than 5 mIU/L. The highest nTSH levels corresponded to neonates of mothers with insufficient ioduria (P = 0.021) or TSH levels greater than 2.5 mIU/L, in both the case of negative (P = 0.049) and positive (P = 0.006) thyroid autoimmunity results. Maternal ioduria greater than 150 µg/L was a risk factor for nTSH levels greater than 5 mIU/L (3.70 [1.06–14.60]; P = 0.046), while a neonatal weight of 2500 g or greater was a protective factor (0.14 [0.02–1.00]; P = 0.038).

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**Introduction**

Iodine is an essential trace element for the synthesis of thyroid hormones. In adults, iodine deficiency is associated with thyroid dysfunction and goitre, and in pregnant women, with increases in the frequency of miscarriage, perinatal mortality and congenital anomalies in the offspring, which in turn is associated with abnormal growth and neurodevelopment. Iodine deficiency continues to be the leading cause of preventable neurologic impairment. The World Health Organization (WHO) recommends universal salt iodization and the use of iodine supplements in at-risk groups, in addition to periodic performance of surveys to monitor iodine status at the population level.

Measurement of the urinary iodine concentration (UIC) in school-age children is the most widely used method to assess population iodine status. However, other markers are also available, such as neonatal thyroid stimulating hormone (TSH) levels, the prevalence of goitre in school-age children or thyroglobulin serum levels.

Neonatal TSH levels are used to screen congenital hypothyroidism in newborns, but it is also a good marker of iodine intake and status, as the low concentration of iodine in the neonatal thyroid gland requires higher rates of iodine turnover, so that TSH levels increase if the iodine supply is low. Based on the recommendations of the WHO, a prevalence of less than 3% of neonatal TSH levels greater than 5 mIU/L is indicative of iodine deficiency in a population. However, numerous obstetric and neonatal factors can influence neonatal TSH levels besides maternal iodine status.

The main aim of our study was to determine the prevalence of neonatal TSH levels greater than 5 mIU/L in our catchment area. A secondary objective was to assess the impact of maternal iodine intake, UIC and thyroid on neonatal TSH levels. Lastly, we analysed other obstetric and neonatal factors that could be associated with neonatal TSH levels.

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**Conclusions:** The prevalence of nTSH levels greater than 5 mIU/L in our health area was high based on the WHO recommendations. Maternal iodine deficiency was associated with a higher risk of nTSH levels greater than 5 mIU/L. Given that nTSH is currently measured before 72h post birth, we need new cut-off points to keep on using nTSH as a marker of iodine nutritional status.

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Material and methods

Study population

We conducted a longitudinal, observational, descriptive and analytic study in pregnant women and neonates in the hospital’s catchment area, with a population of 330,560 inhabitants. Overall, iodine status in this region is adequate, both in the general population and in pregnant women. Participants were selected out of the total of pregnant women who attended the initial visit with the midwife between May and June 2017. The applied inclusion and exclusion criteria can be seen in the flow chart of the study (Fig. 1).

The study was approved by the Regional Research Ethics Committee. All participants signed the informed consent form.

Study variables

In the first visit with the midwife, a questionnaire was administered to assess iodine intake, collecting information on the regular consumption of iodized salt (yes/no) and dairy products (daily servings of milk, yoghurt and cheese), and use of iodine supplementation (yes/no). The results of the iodine intake survey were published by González et al.

Urinary iodine concentration, thyroid function and antithyroid-antibody tests were performed in the first trimester of gestation, the results of which were published by González et al., and we also collected neonatal TSH levels.

The maternal UIC was measured in a random urine sample by inductively coupled plasma mass spectrometry (ICP-MS) with an Agilent 7700x spectrometer (Agilent Technologies, Santa Clara, CA, USA.). This method exhibited an adequate linearity between 10 and 450 μg/L ($R^2 > 0.99$), with an inlababoratory imprecision of 2.9% or less and a total analytical error of 7.3% or less.

In the same visit, the mother underwent collection of a blood sample for measurement of TSH levels and anti-thyroid antibody testing (thyroid peroxidase antibodies [TPOAb] and thyroglobulin antibodies [TgAb]). The measurements were made by electrochemiluminescence immunoassay (Roche Diagnostics, Basel, Switzerland). The coefficient of variation (CV) for TSH values ranged from 0.8% to 2.9%. In our catchment area, the normal range for TSH levels was 0.20–4.50 mIU/L. The normal range for autoantibodies was less than 34 UI/mL for TPOAb and less than 18 UI/mL for TgAb.

Newborns underwent heel lancing, preferably between 48 and 72h post-birth, for collection of capillary blood collected in a Whatman® 903 paper card (dried blood spot) in the framework of the congenital hypothyroidism screening programme. Neonatal TSH was measured by dissociation-enhanced lanthanide fluorescent immunoassay (DELFIA) in the clinical biochemistry laboratory of our hospital. We considered neonatal TSH levels of less than 10 mIU/L normal. If the value was between 10 and 20 mIU/L, a second measurement was made, and in the case of a TSH level greater than 20 mIU/L, the patient was referred to the neonatal unit.

Lastly, we collected data on the birth weight and gestational age of the newborn, mode of delivery (uncomplicated [not requiring intervention by physician/midwife], operative vaginal delivery or caesarean section), reason for...
operative vaginal or caesarean delivery, presence of non-reassuring foetal status (NRFS) and 1-min Apgar score (Apgar-1). The presence of NRFS was determined by the obstetrician if there were any signs or symptoms suggestive of hypoxic metabolic acidosis, including severe clinical conditions and abnormalities in the different diagnostic methods used to assess foetal wellbeing, such as cardiotocography, pulse oximetry, pH measurement or Doppler ultrasound.

Statistical analysis

We conducted a descriptive analysis, calculating absolute and relative frequency distributions for qualitative variables and measures of central tendency and dispersion for quantitative variables. We assessed differences in numerical variables by means of the Student t-test or Wilcoxon test for independent samples based on whether the homogeneity of variance assumption was met. To compare three or more variables, we used analysis of variance (ANOVA) or the Kruskal-Wallis test, based on whether the normality and homoscedasticity assumptions were met. We assessed the association between two variables by means of the $\chi^2$ test or Fisher exact test based on the expected counts. We used the McNemar test in the case of paired proportions. We fitted 2 multivariate binary logistic regression models to predict neonatal levels of TSH greater than 5 mIU/L, calculating odds ratios (OR), 95% confidence intervals (CIs) and $P$ values for the Wald test.

We entered the data in an ACCESS-SQL 2010 database. The statistical analysis was performed with the software R (R Development Core Team), version 36.0. We defined statistical significance as a $P$ value of 0.005.

Results

We selected 332 pregnant women, of who 14 were not eligible because they met 1 or more of the exclusion criteria. Other patients did not complete the study due to miscarriage ($n = 47$), diagnosis of gestational hypothyroidism ($n = 17$) or being lost to follow-up ($n = 11$). Ultimately, we collected data for 243 mother-child dyads.

Out of all neonates, 124 were male. The mean gestational age was 39.8 weeks (standard deviation [SD], 1.6), and 13 infants were born preterm (<37 weeks of gestation). The mean birth weight was $3260 \pm 517.5$ g, and 16 neonates had birth weights of less than 2500 g.

Neonatal TSH levels

Neonatal TSH levels were measured in samples collected between 48 and 72h post birth in 90.4% of newborns (95th percentile [P95], 72h).

The mean neonatal TSH level was 2.43 mIU/L (SD, 1.68). The 5th to 95th percentile range was 0.60–6.60 mIU/L, while the first to 99th percentile range was 0.25–8.60 mIU/L. All newborns had TSH levels in the normal range, and 7.8% had levels greater than 5 mIU/L. Fig. 2 presents the distribution of neonatal TSH levels.

![Figure 2](image1.png)

Figure 2 Histogram of the distribution of neonatal TSH levels.

![Figure 3](image2.png)

Figure 3 Association between neonatal TSH levels and maternal UIC. The bars show the mean neonatal TSH level (shown at top of bar) and the error bars show the standard deviation.

Maternal factors: iodine intake, ioduria and thyroid function

We analysed the association between iodine intake (iodized salt, dairy products and iodine supplements) and neonatal TSH levels, and found no statistically significant differences.

To analyse the association between neonatal TSH and maternal UIC in the first trimester of gestation, we grouped newborns based on whether their mothers had an insufficient (<150 $\mu$g/L), adequate (150–250 $\mu$g/L), greater than adequate (250–500 $\mu$g/L) or excessive (>500 $\mu$g/L) concentration of iodine in urine. The results were statistically significant ($P = 0.021$) and are presented in Fig. 3.
We calculated the proportion of infants with neonatal TSH levels greater than 5 mIU/L based on the maternal UIC, and found statistically significant differences \((p = 0.014)\). The proportion of infants with TSH values greater than 5 mIU/L was 11.9% in the insufficient maternal UIC group compared to 8.3% in the adequate maternal UIC group. All infants born to mothers with an UIC greater than 250 \(\mu g/L\) had neonatal TSH values of less than 5 mIU/L.

The analysis of the association of neonatal TSH values and maternal thyroid function was limited to the cases for which antithyroid-antibody test results were available \((n = 128)\). Table 1 summarises these results. We found that the mean neonatal TSH level was significantly lower in infants born to mothers with TSH values of less than 2.50 mIU/L, independently of thyroid autoantibody status, although the differences were more marked in the subset with mothers who tested positive for thyroid autoantibodies. All infants born to mothers with TSH levels of less than 2.50 and negative thyroid autoantibody test results had neonatal TSH levels of 5 mIU/L or less.

### Obstetric and neonatal factors

#### Type of delivery

Fifty-six percent of infants were born by uncomplicated vaginal delivery, 28.4% by caesarean delivery and 15.6% by operative vaginal delivery. The mean neonatal TSH level was 2.04 mIU/L (SD, 1.52) in the uncomplicated delivery group, 3.04 mIU/L (SD, 1.79) in the caesarean delivery group and 2.88 mIU/L (SD, 1.72) in the operative vaginal delivery group \((p < 0.001)\).

In the uncomplicated delivery group, 4.4% of infants had neonatal TSH levels greater than 5 mIU/L, compared to 7.9% in the caesarean delivery group and 14.5% in the operative vaginal delivery group \((p = 0.039)\).

#### Birth weight and gestational age

We compared neonatal TSH levels in infants with birthweights of less than 2500 g and infants with birthweights of 2500 g and greater, and found no significant differences \((\text{mean} \pm \text{SD}, 2.73 \pm 2.71 \text{ mIU/L vs} 2.41 \pm 1.59 \text{ mIU/L}; \ p = 0.345)\).

We also compared neonatal TSH levels in infants born before and after 37 weeks’ gestation, and also found no significant differences \((\text{mean} \pm \text{SD}, 3.34 \pm 3.17 \text{ mIU/L vs} 2.40 \pm 1.60 \text{ mIU/L}; \ p = 0.224)\).

#### Probability of nonreassuring foetal status

Nonreassuring foetal status was documented in 24.7% of newborns. The proportion of NRFS was 31.2% in infants with birthweights of less than 2500 g compared to 19.8% in those with birthweights of 2500 g or greater. Also, NRFS occurred in 23.1% of infants born before 37 weeks compared to 24.8% of those born at 37 weeks or after. Nonreassuring foetal status was the most frequent reason for caesarean delivery (41.5% of total cases) and operative vaginal delivery (64.2% of total cases).

We compared neonatal TSH levels in infants with a history of NRFS to neonatal TSH levels in infants born by caesarean section or instrumental delivery without NRFS and infants born by uncomplicated vaginal delivery. The mean neonatal TSH values in these groups were 3.22 mIU/L (SD, 1.84), 2.67 mIU/L (SD, 1.75) and 2.02 mIU/L (SD, 1.52), respectively \((p < 0.001)\). Fifteen percent of infants born by caesarean section or operative vaginal delivery due to NRFS had neonatal TSH levels greater than 5 mIU/L compared to 10.4% of infants born by caesarean section or operative vaginal delivery without NRFS and 4.3% of infants with an uncomplicated vaginal birth \((p = 0.026)\).

#### 1-min APGAR

The 1-min Apgar score was less than 9 points in 20.2% of the infants. The neonatal TSH levels in these patients were greater compared to those with 1-min Apgar scores of 9 or more points \((\text{mean} \pm \text{SD}, 2.80 \pm 1.84 \text{ mIU/L vs} 2.34 \pm 1.63 \text{ mIU/L}; \text{although the difference was not statistically significant } (p = .087)\). Of the infants with 1-min Apgar scores of less than 9 points, 16.3% had neonatal TSH levels greater than 5 mIU/L compared to 5.7% of infants with 1-min Apgar scores of 9 points or greater \((p = .031)\). The mean 1-min Apgar score in newborns with neonatal TSH levels of 5 mIU/L or lower was greater compared to newborns with TSH levels greater than 5 mIU/L \((\text{mean} \pm \text{SD}, 8.80 \pm 0.91 \text{ points vs} 7.84 \pm 1.74 \text{ points}; \ p = 0.002)\).

#### Multivariate analysis

We conducted a univariate analysis and fitted 2 multivariate binary logistic regression models (Table 2). The dependent variable in both models was a neonatal TSH level greater than 5 mIU/L. In Model 1, the included variables were maternal UIC, thyroid function and iodide intake. This initial analysis showed that a maternal UIC greater than 150 \(\mu g/L\) was a risk factor for a neonatal TSH level greater than 5 mIU/L \((\text{OR}, 3.27; 95\% \text{ CI, 1.03–11.55}; \ p = 0.049)\). In Model 2, in addition to these variables, we included the mode of delivery, birth weight, gestational age, presence of NRFS and 1-min Apgar score. In this model, a maternal UIC greater than 150 \(\mu g/L\) remained a risk factor for a neonatal TSH level greater than 5 mIU/L \((\text{OR}, 3.70; 95\% \text{ CI, 1.06–14.60}; \ p = 0.046)\), while a birthweight of 2500 g or greater was a protective factor \((\text{OR}, 0.14; 95\% \text{ CI, 0.02–1.00}; \ p = 0.038)\).
A 1-min Apgar score of less than 9 points behaved as a protective factor against neonatal TSH levels greater than 5 mIU/L, with a P value that neared the threshold of significance.

### Discussion

In our study, the mean neonatal TSH level was 2.43 mIU/L, and none of the newborns had TSH levels greater than 10
miU/L. The proportion of newborns with TSH values greater than 5 miu/L was 7.8%. The WHO has proposed that a frequency of 3% or greater of neonatal TSH levels over 5 miu/L is indicative of iodine insufficiency in the population, a criterion that has since been validated by Zimmermann et al. However, pregnant women in our study exhibited an adequate iodine status and the general population in our area is also iodine sufficient.

The main factor that may explain a high prevalence of neonatal TSH levels greater than 5 miu/L in an iodine-sufficient population is the time elapsed from birth to collection of the blood sample. The foetal thyroid starts to synthesise hormones from 18 to 20 weeks of gestation. From that point, TSH levels increase progressively, peaking 30–60 min post birth at values of up to 60–100 miu/L, followed by a marked drop in the next 48 h and eventual stabilisation by 3–5 days post birth. The WHO recommends measurement of neonatal TSH levels in dried blood spots with collection of sample from 72h post birth. However, in our study, as is the case of many other recent ones, samples were collected in the framework of newborn screening for congenital hypothyroidism between 48 and 72h post birth. Our findings corroborate that neonatal TSH levels continue to decline past 72h post birth.

Measuring neonatal TSH levels is the most sensitive method to identify congenital hypothyroidism, but these values may be affected by several factors. One is the maternal iodine status. The foetal thyroid is very sensitive to iodine deficiency, but also to its excess. When iodine levels are excessive, the foetal thyroid is not sufficiently mature to escape the inhibitory Wolff-Chaikoff effect until 36 weeks’ gestation. This is reflected by multiple studies in which excessive iodine intake in the mother was found to be associated with congenital hypothyroidism or the use of iodine-containing antiseptics during childbirth was found to induce transient hypothyroidism in neonates.

In our study, we did not find a significant association between neonatal TSH levels and maternal iodine intake, but we found an association with the maternal UIIC in the first trimester of the pregnancy. The offspring of mothers with sufficient UIICs did not have neonatal TSH levels greater than 5 miu/L and, overall, neonatal TSH levels were higher the lower the maternal UIIC. Both multivariate models showed that maternal iodine deficiency is a risk factor for neonatal TSH levels greater than 5 miu/L, corroborating the association between maternal iodine status and neonatal TSH levels. The results of previous studies have been heterogeneous, with some confirming and others finding no evidence of this association.

Another factor that may have an impact on neonatal TSH levels is maternal thyroid function. In our study, we found that infants born to mothers with TSH levels of less than 2.50 miu/L had significantly lower neonatal TSH levels compared to infants born to mothers with TSH levels above that threshold, with more marked differences in the case of mothers who were positive for thyroid autoantibodies. In addition, all infants born to mothers with TSH levels under TSH <2.5 miu/L and negative for thyroid autoantibodies had neonatal TSH levels of 5 miu/L or less.

In addition to being influenced by the iodine status or thyroid function of the mother, neonatal TSH levels may be affected by multiple maternal, neonatal or obstetric factors resulting in foetal stress. The mode of delivery, preterm birth or low birth weight for gestational age are some of these factors.

Caesarean delivery is associated with lower neonatal TSH levels compared to operative or uncomplicated vaginal delivery. These differences can be explained by the increased catecholamine levels associated with vaginal delivery. Still, there have also been studies that have not found any differences based on the mode of delivery and others in which the highest neonatal TSH levels were found in infants delivered by caesarean section. In our study, infants delivered by caesarean section had the highest neonatal TSH levels, although the multivariate analysis did not find an association between mode of delivery and neonatal TSH levels.

Congenital hypothyroidism is more frequent in preterm or low birth weight newborns due to the immaturity of the hypothalamic-pituitary axis. Preterm infants may experience transient hypothyroxinaemia in the first weeks of life, with a delayed postnatal TSH peak that usually takes place 2–6 weeks post birth. Low birth weight is also an independent factor that may influence neonatal TSH levels. In our study, a birth weight greater than 2500 g was associated with a lower probability of neonatal TSH levels greater than 5 miu/L, although we did not find an association with gestational age.

Lastly, we analysed 2 factors that are direct indicators of foetal stress, such as non reassuring foetal status and the 1-min Apgar score. Neither of these factors was significantly associated with TSH levels in our study, but Korevaar et al. found that factors related to foetal stress, such as respiratory distress, were associated with transient abnormalities in neonatal thyroid function. Along the same lines, other studies have found an association between foetal distress or low Apgar scores and abnormal thyroid function in the neonatal period.

The main limitation of our study was the sample size which, while large enough to assess the primary outcomes, was too small to yield statistically significant results in some of the analyses. Another limitation was the retrospective data collection, as there could have been confounding factors at play that were not taken into account and interfered with the results.

In conclusion, the prevalence of neonatal levels of TSH greater than 5 miu/L in our catchment area was high based on the recommendations of the WHO, in spite of being a population with an adequate iodine status. Neonatal TSH levels are a good marker of iodine nutritional status, as demonstrated by their correlation with maternal urinary iodine concentrations in the first trimester of gestation, and maternal iodine deficiency was associated with an increased risk of neonatal TSH levels greater than 5 miu/L. However, since neonatal TSH levels are currently measured within 72h of birth in the context of screening for congenital hypothyroidism, new cut-off points need to be established to continue using this parameter as a marker of iodine status, which requires performance of additional studies.

**Conflicts of interest**

The authors have no conflicts of interest to declare.
References