Ultrasonographic description of brain cortex and cingulate sulcus development in Mexican neonates and infants with congenital hypothyroidism

Gerardo A. Alvarado-Ruiz1,4,* , Carmen Sánchez-Pérez1, Eugenio Morales2, Mario Mandujano1, Martínez-Vázquez Rosa Ivone1, María de la Luz Ruiz-Reyes3 and Raúl Calzada-León3

1 Follow-up Neurodevelopment Laboratory, Instituto Nacional de Pediatría and Universidad Autónoma Metropolitana Xochimilco, México City, México
2 Radiology Department, Instituto Nacional de Pediatría, México City, México
3 Endocrinology Service, Instituto Nacional de Pediatría, México City, México
4 CMF Tlalpan ISSSTE, México City, México

Abstract

Background: Ultrasonography of cortical and cingulum maturity patterns, were studied in newborns and infants with congenital hypothyroidism (CH).

Method: Transversal study of 29 newborns and infants with CH, detected by neonatal screening and confirmed with thyroid function test, thyroid ultrasonography, and thyroid scintigraphy. During the first 2 months of life, transfontanelar brain ultrasonography was performed. Brain cortex maturity was assessed by normality referents provided by Slagle and Timor methods.

Results: Cortical immaturity signs were observed in 69% of infants (20 patients with Slage’s method brain cortex development delay (Pearson’s p=0.05). Logistic nominal analysis for normality prediction demonstrated a correlation between brain cortex development and age, bone age, treatment duration, and type of CH. The most sensitive detecting technique was sagittal sight by Slagle’s method.

Conclusions: Brain cortex delayed development is frequent in children with CH. Bone age, postnatal age at treatment start, and time since treatment start, correlates with neurodevelopmental delay (Pearson’s p = 0.05). Logistic nominal analysis change for at least the first 2 months of postnatal life (1–6).

Although thyroid hormone production in the fetus begins at 12 WG, and increases though 40 WG, it is insufficient to reach functional concentrations; maternal thyroid hormones crossing the placental barrier are necessary to maintain normal triiodothyronine (T3) and thyroxin (T4) concentrations during the pregnancy and to assure osseous and neurological development of the fetus (neuronal migration and synaptogenesis, as well as osteoblast and chondroblast maturation, with a peak at 20–24 WG). Consequently, if thyroid hormones production during fetal life, the maternal transference of thyroxin, or both, were deficient, there will be a great risk of delayed central nervous system development (including alterations in cerebral cortex maturation and organization) and bone maturation delay (7–17).

Several changes have been reported in cortex development of hypothyroid animals, such as delayed development of the neuropil, deficient cell proliferation, migration and myelination, mainly in the visual and auditory cortex, hippocampus and cerebellum. All are proportional to the duration and severity of hormonal deficiency during pregnancy (13).

Transfontanelar cerebral ultrasonography represents a complementary diagnostic method for different illnesses affecting the cerebral cortex. Tridimensional ultrasonographic equipment with high-resolution imaging in real time shows stages in cerebral cortex maturation, based on structural changes that appear progressively on the inner surface of the brain, mainly the development of fissures that appear at different times, such as the choroidea at 13 WG, the pericallosal around 26 WG, and the callosal marginal or cingulate at 28 WG. The latter described for its associations with the corpus callosum and inner circulation of the brain, is an important marker of both cortical maturation and congenital alterations, and is very useful in perinatal encephalopathies (leukolamacia, infarct, etc.).
Among the ultrasonographic studies that have explored cerebral cortex maturation to determine gestational age (18-21), one of the most useful is the work carried out by Slagle et al. (20) in 211 healthy newborns from 24 to 40 weeks of gestation, that in sagittal sections of transfontanellar ultrasonography, shows the development of the cingulate sulcus and the presence of primary, secondary and tertiary sulci. His paper reports deformities or delays in cingulate circumscription and its relationship with lesions in the immediate vicinity. The maturational organization of these sulci has also been reviewed in coronal projections by the Timor-Trisch et al. (22, 23). This study used transvaginal ultrasound in fetuses during the last trimester of gestation, and reports that circumscriptions and main sulci become prominent and deep in the last trimester of gestation, leading to secondary and tertiary circumscriptions according to a known developmental pattern.

Other authors have studied cerebral cortex maturation to estimate fetal age (24, 25) and have reported associations between cerebral maturation and the neurodevelopment of normal children. Likewise, it has been suggested that genetic or congenital defects may cause cortex maturation delay, which would be expressed by delayed behavior or early neurological signs that will affect the scholar knowledge due to learning difficulties (26-29).

Knowing the participation of thyroid hormones in overall development, specifically in cerebral maturation during the perinatal period, and the absence of ultrasonographic studies describing the relationship between cortical maturation and CH presence, we decided to initiate this prospective study. In Mexico, there is a high prevalence of congenital hypothyroidism (CH) ranging from 4.12 to 5.95/10,000 live births; in Mexico City (30, 31).

This research describes newborns and infants 0 to 2 months of age with a confirmed CH diagnosis via the Slagle and Timor ultrasonographic methods, and determines the predictive value of these cortical maturation patterns with the CH type.

Materials and methods

A cross-sectional, prospective study with Investigation and Ethical Committees approval was performed in 29 children with CH, referred to the Follow-up Neurodevelopment Laboratory (FNL) during the first two months of life. The patients were included because of hypothyroidism suspected by neonatal screening and confirmed by thyroid function test (chemiluminescence, and Tc99-scintigraphy). Jurado García tables for Mexican newborns (32, 33) were used to characterize the birth condition. Knee bone age was evaluated in all (Pyle method) to determine skeletal maturation. In all cases, informed written consent was obtained from the parents to perform the ultrasonography. Children with intrauterine delay of growth, low Apgar score at birth, genetic malformations or other associated congenital alterations or who were small for gestation age, were excluded.

Ultrasonography studies were carried out using a portable Medison C. Ltd equipment SA-6000 model (Korea Data System Co Ltd, Seoul, Korea) with a low, medium and high frequency transfontanellar ultrasound (3, 5 and 7 MHz) for deep, medium and superficial planes. The subject was in the supine position, in a functional sleep state or while feeding, and held by one family member. After coating with gel on the anterior fontanel, three coronal sections (anterior, mid and posterior) and three sagittal sections (left, mid and right) were explored, evaluating the developmental characteristics of the cingulate sulcus and the presence of primary, secondary and tertiary sulci. The patterns of cerebral cortex maturation were rated with the normality standards for newborns and infants provided by the Slagle and Timor methods (Figure 1). The cingulate was identified in the parasagittal planes between the thalamus and the anterior fontanel. The tests were video recorded in 8 mm format, digitalized for computer analysis and graded by two experts; neither assessor was aware of the background.

The results are submitted firstly in descriptive form for the demographic variables: gestational age (GA), weight at birth (BW), length at birth (BL), and cranial perimeter (CP). The clinical variables were hypothyroidism type (TCH), thyroid hormone levels at diagnosis (TH), bone age (BA), chronological age at the start of hormonal treatment (ATX), days of treatment at the time of the (ultrasonographic) USC test (DTX) and results from maturational ultrasound age of the cerebral cortex from Slagle (USCS) and Timor (USCT). A variance analysis and logistic regression of the maturational condition of the cerebral cortex (USCS) and (USCT) were performed in relation to the age at start of treatment (ATX) and days of treatment (DTX), and contingency analysis, correspondence analysis, and χ2 calculation with demographical (GA, BW, BL, CP) and clinical variables (TCH, TH, BA).

The variables TCH, TH, BA and ATX, and TCH, BA and CP were clustered using the conglomerate method by the Ward’s hierarchical distance procedure and associations of resulting typologies with cortical maturation patterns were analyzed. Prediction of normality and immaturity patterns was carried out with nominal logistic analysis for the BA, TH, ATX, DTX model and a variance analysis for each of these variables. The contouring or curve graphs method was used to investigate the effect of time and age at the start of treatment.

The SAS, version 7.0 (SAS Statistical discovery software, North Carolina, USA) JMP statistical program was used to prepare the results tables and graphs.

Results

The mean values for demographic variables were: GA 40±1.7 weeks, BW 3104.85±594 g, BL 49.4±2.42 cm; CP was normal in 24 cases and below percentile three in four cases. Twenty-four cases were females and five were males, with a 4.8:1 ratio.

Sublingual nodule/node (SLN) was found in 14 and athyreosis (AT) in 15 children. Serum concentrations of total T3 and free T3 showed a significant difference by type of CH (Table 1).

The time for diagnosis confirmation and treatment start was 34±11 days (10–57 days), and the lapse between treatment start and USC performance was 12±10 days (1–35 days). Delayed bone age was present in 21 children (AT=13, SLN=8). All cases with cranial perimeter below percentile 3, showed delayed bone age; one boy with SLN and four girls with AT.

According to the USCS method, 20 children had cerebral cortex immaturity; two with type 3 (32–34 GW) and 18 with
Hyperechoic echoes zone pattern obtains higher amplitude and deepness for both level of cingulum as other sulci from brain cortex. Several zones of hyperechoic echoes corresponding to secondary branches that are added to cingulum circvolution.

Presence of one or two discontinuous lineal echoes in the cingulated region between the thalamus and the anterior fontanelle.

The first indention of cingulated sulcus appears. Cingulated sulcus indentation is observed, and new indentations along longitudinal cisure appear.

Longitudinal fissure is broader and deeper and there is development of secondary and tertiary sulci.

Bigger amplitude of the longitudinal cisure and the cingulated sulcus is observed with longitudinal development of new circumvolutions.

The study identified sublingual nodule (SLN) in 14 children, athyreosis condition (AT) in 15 children. Total and free T3 serum concentrations showed a significant difference among different types of hypothyroidism.

<table>
<thead>
<tr>
<th>Athyreosis</th>
<th>Sublingual nodule</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Maximum value</strong></td>
<td><strong>Minimum value</strong></td>
</tr>
<tr>
<td>T3T ng/dL</td>
<td>73.0</td>
</tr>
<tr>
<td>T3L pg/mL</td>
<td>2.63</td>
</tr>
<tr>
<td>T4T μg/dL</td>
<td>2.7</td>
</tr>
<tr>
<td>T4L ng/dL</td>
<td>0.4</td>
</tr>
<tr>
<td>TSH μU/mL</td>
<td>100.0</td>
</tr>
<tr>
<td>TG ng/dL</td>
<td>97.2</td>
</tr>
</tbody>
</table>

Pearson’s χ² p<0.05.
type 4 (35–38 GW). The USCT method demonstrated 17 children with immaturity patterns; 1 type 2 (33–35 GW) and 16 type 3 (36–37 GW) (Figure 2).

The correlation between the Slagle and Timor methods shows 23 coincidences (9 cases with normal maturity and 14 cases with delayed maturation). The discrepancies seen in six cases were because USCS classified two cases as type 3 (32–34 GW) but USCT as type 3 (36–37 GW); three cases were type 4 (35–38 GW) according to USCS but type 4 for USCT (38–39 GW) and one patient corresponded to type 4 (35–38 GW) for USCS but to type 2 for USCT (33–35 GW).

The contingency analysis showed a direct relationship between bone age and USCS or USCT method for cerebral cortex maturation, being significant with the USCT method (Pearson’s $\chi^2 p=0.05$) (Figure 3).

Variance analysis for ATX for USCS was $33\pm7.6$ vs. $36\pm15.9$ days for immature vs. normal children, and DTX $9\pm8.25$ vs. $17\pm10.69$ days for immature vs. normal children, respectively. For the USCT method, ATX was $33.05\pm6.7$ vs. $35.2\pm15.5$ days, and DTX $10\pm8.87$ vs. $13\pm11.01$ days for immature vs. normal children, respectively.

To profile the severity of the hypothyroidism, we proceeded to form clusters with the clinical variables that were associated or showed tendency to be associated with the immaturity patterns through Ward’s hierarchical distance procedure.

Two typologies grouped by TCH (AT or SLN), plasma levels of thyroid hormones, BA and ATX were obtained from the first cluster analysis. The first typology “light disease” was formed in 12 cases with SLN, normal BA and normal levels of TH; the second or “severe disease” was formed in 17 cases, including the patients with AT, delayed BA and low levels of TH (Tables 2 and 3).

The association of the two typology clusters with a cerebral cortex maturation state was explored using contingency, $\chi^2$ and correspondence analysis; significant marginal association was found between the USGS and severe cases (typology 2) with more immature cases (32–34 GW), while mild cases (typology 1) correlate with mature cases (38–40 GW), obtaining a Pearson’s $\chi^2$ of 0.06. No meaningful association was found with the USCT method (Figure 4).

In order to determine the probability of prediction of cerebral cortex normality and immaturity patterns with the USCS and USCT methods, we used a nominal logistic model with BA, TCH, ATX and DTX as independent variables, considering maturity or immaturity as dependent variables. For USCS, the complete model was significant with a $\chi^2=15.54$ ($p<0.0029$), and the adjusted model demonstrated significant association with BA ($p<0.03$), ATX ($p<0.02$) and DTX ($p<0.02$). For USCT, the complete
model had no significance, but the adjusted model found an association with BA ($\chi^2 = 4.47, p < 0.03$), and with a contouring graph or level curve; the associated probability of normal cortex maturation increased to 0.8 when ATX and DTX values were ≤15 days, but when ATX was >48 days, DTX was 2 days (Figure 5).

Discussion

GA, BW and BL were similar to the Mexican general population (32, 33) and also for growth in children with congenital hypothyroidism detected by neonatal screening (34). They indicated that a difference in growth expression is observed only if treatment start is prolonged beyond 120 days. In our research, 11 children (38%) started treatment at <1 month of age, 16 (58%) between 31 and 50 days, and 2 (7%) between 51 and 57 days.

We found differences in TH at diagnosis (mainly T3 and FT3) according TCH, with lower levels in AT and a direct relationship between TH and BA in 88% (13/15). It is well known that neonatal TH, mainly T3, has a substantive impact on long-term behavior, motor skills, language, hearing and cognitive development, and previous studies have shown the importance of factors, such as the TCH, ATX, and intra-uterine HT in the development of the CNS since the 2nd half of gestation and their impact on psychomotor development. In Holland, the children of 220 mothers with low hormonal serum concentration showed rates below the 10th percentile in their psychomotor development at 10 months of age (35–38).

MRI and ultrasound studies had described the association between brain cortex maturation and psychomotor development and some authors propose that cortical maturation is equivalent to GA (6, 20–23, 39–42).

MRI and spectrographic analysis show reductions in N-acetylaspartate and choline, confirming progress of the cortex maturation and myelination in newborns with CH, as well as late in development and the number of secondary and tertiary sulci in patients with cortical dysplasia (43, 44).

In this study, children with CH had a high percentage (69%) of mild or moderate delay in cerebral cortex maturation. A group of four cases with very low CP stood out; all had generalized immature patterns due to deficient differentiation of secondary and tertiary sulci and nearly uniform cingulate. One had cortical immaturity Slagle type 3 (33–35 GW), and Timor type 2 (36–37 GW), and three had cortical immaturity Slagle type 4 (35–38 GW), and Timor type 3 (36–37 GW). All four presented AT and delayed BA, and although neonatal hypoxia can not be ruled out, none had lesions characteristic of acute or sub acute perinatal encephalopathy, such as ventriculomegaly, asymmetrical subarachnoid dilation or deep or extended sulci.

The value of perinatal encephalopathies detected by ultrasound, as a prognostic determinant of psychomotor development during childhood, has been demonstrated by several authors, and it is applicable even for patients with CH (44–49).

<table>
<thead>
<tr>
<th>Cohort variables</th>
<th>Type 1 mild (n=14)</th>
<th>Type 2 severe (n=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gammmigraphic alteration of the thyroid</td>
<td>12 SLN</td>
<td>2 SLN</td>
</tr>
<tr>
<td></td>
<td>0 Athyreosis</td>
<td>15 Athyreosis</td>
</tr>
<tr>
<td>T3l pg/mL</td>
<td>Rank 2–5</td>
<td>Rank 1–2.63</td>
</tr>
<tr>
<td></td>
<td>Median 3.90</td>
<td>Median 1.2</td>
</tr>
<tr>
<td>T3t ng/dL</td>
<td>Rank 93–188</td>
<td>Rank 0.26–73</td>
</tr>
<tr>
<td></td>
<td>Median 147</td>
<td>Median 40</td>
</tr>
<tr>
<td>T4l ng/dL</td>
<td>Rank 0.2–1.1</td>
<td>Rank 0.02–0.5</td>
</tr>
<tr>
<td></td>
<td>Median 0.5</td>
<td>Median 0.2</td>
</tr>
<tr>
<td>T4t μg/dL</td>
<td>Rank 1.6–8.2</td>
<td>Rank 0.04–2.7</td>
</tr>
<tr>
<td></td>
<td>Median 4.15</td>
<td>Median 1</td>
</tr>
<tr>
<td>Days of initiated the hormonal substitutive treatment</td>
<td>Rank 0–35 days</td>
<td>Rank 0–27 days</td>
</tr>
<tr>
<td></td>
<td>Median 4.5 days</td>
<td>Median 15 days</td>
</tr>
<tr>
<td>Bone age</td>
<td>6 without delay in bone age</td>
<td>2 without delay in bone age</td>
</tr>
<tr>
<td></td>
<td>6 with delay in bone age</td>
<td>15 with delay in bone age</td>
</tr>
</tbody>
</table>
This study demonstrated a high percentage of hypothyroid neonates and infants with ultrasound signs of cortex immaturity, and suggests that all patients with CH must be evaluated with Slagle’s method for cerebral transfontanelar ultrasonography at the moment of diagnosis, to determine the risk of neurological immaturity that will compromise the psychomotor development, particularly in those with athyreosis, low plasma levels of thyroid hormones, delayed bone age, and mother’s thyroid disease during pregnancy, all of which are suggestive of intrauterine deficit of thyroid hormones.

It could explain the observations that even with an early start of treatment, there are learning and attention problems at school age, quite probably as the result of cerebral cortex alterations (50).

Severity of damage in psychomotor development during infancy and childhood in patients with CH, has been associated with athyreosis and delayed start of treatment, but according to our results, we suggest that the finding of ultrasound cerebral immaturity should also alert to the need to initiate early preventive and intervention actions in the program of neurodevelopment follow-up, mainly in patients with delayed bone age at diagnosis, as we demonstrated that this is an additional factor for neurological immaturity and that athyreosis did not have a significant association with brain cortex maturity.

**Conclusions**

Slagle’s ultrasound method detected the greatest number of infants with cerebral maturation delay and it was associated through cluster analysis with cases of athyreosis, delayed bone age, and low serum levels of thyroid hormones; in conjunction with TCH, ATX and DTX, it can significantly predict immaturity patterns with USCS but not with USCT.
We propose conducting follow-up studies on these infants, with the aim of learning the prognostic value of these ultrasound patterns at preschool and school age.

References


