ORIGINAL ARTICLES

The impact of gestational age at birth on thyroid function; does it influence the screening protocol for congenital hypothyroidism?

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ABSTRACT

Aim: to demonstrate the impact of gestational age at birth on thyroid function and also to determine the validity of a repeat thyroid function test in screening for congenital hypothyroidism (CH) for preterm neonates.

Methods: A total of 280 appropriate for GA neonates (premature or sick full term) between 28-41 weeks gestation admitted to Al Galaa teaching hospital were enrolled in the study. The study was prospective and had been carried out in the period between June 2011 and December 2012. TSH and T4 were measured in blood between 6-7 days (test 1) and once again 4-5 weeks after birth (test 2). In test 2 samples were only drawn from 209 neonates as 3 neonates died in the period between the first and second test while the remaining were missed during follow up. The studied population were divided into 3 gestational age groups; group A (28-31 weeks), group B (32-36 weeks) and group C; control (≥37) weeks. The mean gestational age, birth weight and other clinical data were determined for each group. Results: In test (1), our results showed significantly lower plasma concentrations of T4 and TSH in premature than in term babies while in test (2), values were within the normal range in all neonates included in the study. This study showed that 48% of preterm infants had transient hypothyroxinemia on day 6-7 (test 1) which was corrected spontaneously by day 28-35 (test 2). Gestational age and birth weight were significantly lower in preterm infants with hypothyroxinemia than in preterm infants without hypothyroxinemia (p<0.01). Hypothyroidism was diagnosed in 10 infants, about (6%). Preterm infants with hypothyroidism also had significantly lower gestational age and birth weight than control (p<0.01). In test (2) TSH and T4 attain normal values. There were a significant difference in serum T4 values and serum TSH when comparing group A (28-31ws) with other groups (B&C), but this difference was found to be non-significant when group B&C are compared with each other (test1). The maximum effect of gestational age and birth weight on thyroid function was on group A. TSH levels had a tendency to increase in test 2 in all groups. No cases with delayed TSH rise were detected in this study. Conclusion: Premature infants especially those born before 31 week gestations, had a high incidence of transient thyroid dysfunction (hypothyroxinemia and less commonly hypothyroidism). Up till now the optimum screening protocol of congenital hypothyroidism for preterm neonates is not settled. In this study all infants with low T4 and TSH attains normal values after 4-5 weeks (test 2), also there were no cases with delayed TSH rise diagnosed. So we believe that routine second screening test may not be needed due to increased costs with a very low yield of cases and to limit repeat screening to selected newborn populations (including preterm especially those with gestational age below 31 weeks and sick newborn) in whom the incidence of hypothyroxinemia and hypothyroidism are high.

Key words: Newborn screening, Preterm, TSH, T4

Introduction

The development of the normal fetal-neonatal thyroid system can be categorized in three phases. The first one begins with thyroid and pituitary embryogenesis occurring up to the 10th-12th weeks of gestation. The histologic and functional maturation of the hypothalamus and of the pituitary portal vascular systems begins at 4th-5th gestational weeks and continues through gestational weeks 30-35. The third and final phase of fetal thyroid development is the maturation of the hypothalamic-pituitary-thyroid axis beginning at mid-gestation and continuing through to approximately 4 weeks postnatally. One can easily infer that infants born before term may have disruption in the normal maturation of the fetal hypothalamic-pituitary-thyroid axis leading to abnormal thyroid function. (De Felice et al., 2004 and Kratzsch et al., 2008).

Congenital hypothyroidism is one of the most common preventable causes of mental retardation. Its clinical features are often subtle and many newborn infants remain undiagnosed at birth (LaFranchi, 1979 and Kaplan, 1990). The slow development of obvious clinical symptoms (ALm et al., 1984), coupled with the importance of
early treatment led to the implementation of widespread newborn screening for this condition (Fisher, 1983). There are different screening strategies. The most popular is early screening which is adapted by most centers. However; some centers apply a second delayed screening to detect what is called delayed TSH rise. The value of this strategy has not yet proved. The Northwest Regional Newborn Screening Program, which has performed a routine second screening test since inception, reported detection of infants with congenital hypothyroidism associated with delayed TSH rise, with an overall incidence of 1:67,226 (Hunter et al., 1998). Most investigators ascribe the cause of delayed TSH rise to immaturity of the hypothalamic-pituitary thyroid axis. However, "immaturity" of the hypothalamic-pituitary-thyroid axis is disputed by the fact that most cases of permanent, primary congenital hypothyroidism in premature infants are detected by results from the initial newborn screening test (Fisher, 2007).

It is known that prematurity complicates screening for primary hypothyroidism due to developmental delay in the maturation of the hypothalamic-pituitary-thyroidal axis, and different methodological approaches have been proposed so that no preterm newborn escapes early diagnosis for congenital hypothyroidism, i.e. repeat specimen collection (Mitchell et al., 1994a; Mitchell et al., 1994b). Several screening protocols for the detection of CH have evolved: a primary TSH/backup T4 method and a primary T4/backup TSH method. In addition, an increasing number of programs use a combined primary TSH plus T4 approach. Most programs in Europe, Japan, Canada, Mexico, and the United States screen by using primary TSH measurements, supplemented by T4 determinations for infants with elevated TSH values (Rose et al., 2006).

The aim of this study was to: demonstrate the effect of gestational age at birth on thyroid function and to determine the validity of a repeat thyroid function test in screening for CH for preterm neonates.

Materials and Methods

A total of 280 appropriate for GA neonates (premature or sick full term) between 28-41 weeks gestation admitted to Al Gala teaching hospital were enrolled in the study. Full term neonates were selected from labor wards while preterm neonates were selected from neonates admitted to Neonatal Intensive Care Unit (NICU). The study was prospective and had been carried out in the period between June 2011 and December 2012.

Gestational age was assigned by early obstetrical ultrasound (US) and last menstrual period (if sure). If these data are not available, gestational age was assessed by neonatologist attending delivery or immediately after delivery. Newborn infants born before completing 37 weeks of gestation were considered premature. Exclusion criteria from the study were: major congenital anomalies, infants with maternal thyroid diseases or maternal history of taking antithyroid drugs, clinically significant evidence of infection, necrotizing enterocolitis, interventricular hemorrhage and inability of mother to provide informed consent. All infants had intensive care support as required, including: intermittent positive pressure ventilation, surfactants supplementation, and correction of fluids, electrolytes, blood glucose and acid base abnormalities. Written informed consents were obtained from all patients as well as an institutional board approval before starting the study.

Sampling:

Two samples were drawn for each subject, 2 ml for each, TSH and T4 were measured in blood between 6-7 days (sample 1) and once again 4-5 weeks after birth (sample 2). In sample 2, samples were only drawn from 209 neonates as 3 neonates died in the period between the first and second test while the remaining were missed during follow up.

If an umbilical arterial or venous catheter was already in place, blood was withdrawn from it otherwise 1 ml samples were obtained by venipuncture at the time blood for other routine laboratory work was drawn. Blood collected into a tube without anticoagulant and allowed to clot for at least 15 minutes and then centrifuged.

Clinical data were retrospectively analyzed in terms of gestational age, birth weight, Apgar score at 5 minutes and the development of RDS and the need for surfactant supplementation. Information regarding prenatal treatment with dexamethasone is considered and the use of dopamine on the day on which the thyroid function tests were included in the study.

The studied population were divided into 3 gestational age groups: group A (28-31 weeks), group B (32-36 weeks) and group C; control (≥ 37) weeks. The mean gestational age, birth weight and other clinical data were determined for each group.

Laboratory test:

Quantitative determination of TSH and T4 by VIDAS (supplied by Biomerieux); The assay principle combines an enzyme immunoassay sandwich method with a final fluorescent detection (ELFA). The solid phase receptacle (SPR) serves as solid phase as well as the pipetting device for the assay. Reagent for the assay is
ready to use and pre-dispensed in the sealed reagent strip. The sample 200µl was taken and transferred into the well containing alkaline phosphatase labeled with anti TSH,T4 antibodies, the sample and conjugate mixture is cycled in and out the SPR several times to increase the reaction speed. The antigen binds to antibodies coated on the SPR and to the conjugate forming a sandwich; unbound components are eliminated during the washing step. During the final step the substrates (4- methyl- umbelliferone phosphate) is cycled in and out of SPR. The conjugated enzymes catalyzes the hydrolysis of the substrate into fluorescent product (4- methyl-umbelliferone), the fluorescence of which is measured at 450 nm, the intensity of fluorescence is proportional to the concentration of antigen present in the sample at the end of the assay. The results are automatically calculated by the instrument in relation to calibration curve stored in the memory and then printed out.

The normal TSH value was defined as less than 15mIU between the third and six days after birth (test 1) and less than 10 mlU at four week of age (Dorota et al., 2005). Normal reference value of T4; at 1-2 weeks is 126-214nmol/L and 93—189 nmol/L at 1-2month(Whitley, 1999). Hypothyroxinemia of prematurity was defined as low T4 level with TSH level < 7 uU/ml in the test (1). Hypothyroidism was defined as low T4 level in conjunction with TSH levels ≥ 10uU/ml or as TSH level ≥ 30uU/ml in conjunction with any level of T4 (Dorota et al., 2005).

Data were expressed as mean, standard deviation (SD) and percentage as appropriate. Groups were compared using t test or the chi square test as appropriate. P value less than 0.05 was considered significant.The statistical analysis was done using SPSS version 10 statistics program.

**Results:**

The clinical characteristics of the study infants were summarized in table (1). The neonates were divided into 3 gestational age groups for data analysis; Group A (neonates with gestational age ranging from 28 to 31weeks), Group B (32-36 weeks), Group C (Control group; 37-41 weeks).

**Table 1:** Clinical characteristics of the study infants.

<table>
<thead>
<tr>
<th></th>
<th>Preterm</th>
<th>Term≥37</th>
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<tbody>
<tr>
<td></td>
<td>Group A (n=79)</td>
<td>Group B (n=91)</td>
</tr>
<tr>
<td>Gestational age in completed weeks (mean±SD)</td>
<td>29.9±2.6 (28 – 32 ws)</td>
<td>34.1±1.4 (33 – 36 ws)</td>
</tr>
<tr>
<td>Birth Weight (kg) (mean±SD)</td>
<td>1190±11</td>
<td>1970±51</td>
</tr>
<tr>
<td>Caesarean section (n=129)</td>
<td>61 (77%)</td>
<td>54 (59%)</td>
</tr>
<tr>
<td>RDS (n=46)</td>
<td>32 (40%)</td>
<td>12 (13%)</td>
</tr>
<tr>
<td>Apagar score (5 minute) mean ±SD</td>
<td>5.1±1.9</td>
<td>6.2±1.4</td>
</tr>
<tr>
<td>Prenatal treatment with corticosteroids (n=110)</td>
<td>58 (73%)</td>
<td>61 (68%)</td>
</tr>
<tr>
<td>Sex ; Female (n=110)</td>
<td>42 (53%)</td>
<td>49 (54%)</td>
</tr>
<tr>
<td>Male (n=99)</td>
<td>37 (47%)</td>
<td>42 (46%)</td>
</tr>
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</table>

Since we assessed infants on the 6-7 days of life our results are not affected by factors such as mode of delivery, fetal distress, and prenatal steroid or neonatal surfactant use. However, it was observed that preterm infants with thyroid abnormalities (hypothyroxinemia and hypothyroidism)had significantly lower gestational age and birth weights compared to preterm infants with normal thyroid functions. This study showed that 48% of preterm infants had transient hypothyroxinemia and 6% had transient hypothyroidism on day 6-7 (test 1) which were corrected spontaneously by day 28-35 (test 2). These data are shown in table (2)

**Table 2:** Comparison between preterm infants with Hypothyroxinemia or hypothyroidism and those without. (control).

<table>
<thead>
<tr>
<th></th>
<th>Preterm with Hypothyroxinemi n=62</th>
<th>Preterm with Hypothyroidism n=10</th>
<th>Control n=98</th>
<th>P1</th>
<th>P2</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA (week)</td>
<td>28.4±19</td>
<td>29.9±18</td>
<td>32.6±17</td>
<td>&lt;0.01(S)</td>
<td>&lt;0.05(S)</td>
</tr>
<tr>
<td>CS</td>
<td>79%</td>
<td>74%</td>
<td>75%</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Apgar score 5</td>
<td>6.4±1.8</td>
<td>6.1±2.1</td>
<td>6.2±1.6</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Birth weight</td>
<td>1015±191</td>
<td>1190±211</td>
<td>1279±291</td>
<td>&lt;0.01(S)</td>
<td>&lt;0.01(S)</td>
</tr>
<tr>
<td>RDS</td>
<td>76%</td>
<td>64%</td>
<td>62%</td>
<td>&lt;0.05(S)</td>
<td>&lt;0.05(S)</td>
</tr>
<tr>
<td>Surfactant</td>
<td>29%</td>
<td>33%</td>
<td>24%</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

GA; Gestational age,RDS; Respiratory Distress Syndrome, CS; Cesarean Section, N S; None Significant.S; significant.Control; Preterm without Hypothyroxinemia or hypothyroidism.P1; Preterm with Hypothyroxinemia and Control, P2; Preterm with Hypothyroidism and control. P value less than 0.05 was considered significant.

RDS was diagnosed in 44 preterm neonates (36 of them had hypothyroxinemia, (about 82%) and in only 2 term neonates. The incidence of RDS was significantly greater in infants with hypothyroxinemia than in infants without hypothyroxinemia (p<0.05).

In early screening (test1), our results showed significantly lower plasma concentrations of T4 and TSH in premature than in term babies (p<0.001),while in test (2), all the hormonal levels were significantly increased
and attained normal values (values were within the normal range in all neonates included in the study) i.e., the difference was not significant upon delayed screening.

<table>
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<th>Table 3: Comparison between T4 and TSH levels in preterm and term infants in test 1 and test 2.</th>
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<td></td>
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<tr>
<td>T4 (nmol/l)</td>
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<tr>
<td>TSH (mU/l)</td>
</tr>
</tbody>
</table>

Unpaired ‘t’ test was done between preterm and term in test 1 and 2. P value less than 0.05 was considered significant.

There were a significant difference in serum T4 values and serum TSH when comparing group A (28-31 wks) with other groups (B&C), but this difference was found to be non-significant when group B&C are compared with each other (test1). The maximum effect of gestational age and birth weight on thyroid function was on group A. Most infants born < 31 week gestation (group A) had low T4 concentration in test 1 (6-7 days). Serum levels increase to reach levels equal to those of term infants in test 2 (28-35 days). TSH levels had a tendency to increase in test 2 in all groups.

<table>
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<th>Table 4: Comparison between T4 and TSH levels in groups A, B and C in test 1 and test 2.</th>
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<td></td>
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<tr>
<td>Group C (Control): n=38</td>
</tr>
<tr>
<td>Group A (28-31): n=79</td>
</tr>
<tr>
<td>Group B (32-36): n=91</td>
</tr>
</tbody>
</table>

‘t’ test was done between C & A , C&B and A&B; In test (1), P1; between groups A&C, P2; between groups B&C, P3; between groups A&B. In test (2), P1*; between groups A&C, P2*; between groups B&C, P3*; between groups A&B. P value less than 0.05 was considered significant.

We compared TSH and T4 levels in the 3 groups in test 1 and test 2. In test 1, group A (extremely premature infants) had significantly lower T4 and TSH levels when compared to controls (group C) (p<0.05) as well as when compared with group B (p<0.05). There were no significant differences when group B and C were compared with each other. TSH, in test 1, was lower in groups A and B compared to controls however, the difference was not statistically significant. These data are shown in table 4. In delayed screening (test 2), T4 and TSH levels attained nearly normal values so that there was no statistically significant difference between individual groups.

Discussion:

Late second and early third trimester are critical transition periods in fetal thyroid hormone metabolism, which may be interrupted by preterm birth and contribute to postnatal thyroid dysfunction (Hume et al., 2004). The incidence of thyroid dysfunction in preterm infants was higher than control, pituitary feedback for thyroid hormone are limited and TSH may not be increased even though serum thyroid hormones is low, this could be attributed to developmental delay in the maturation of the hypothalamic-pit-thyroidal axis (Franklin et al., 1986). Thyroid hormones are associated with the neurodevelopment of preterm infants (Redding et al., 1974 and Klein et al., 1977). Despite many studies on thyroid function in preterm infants, its significance is still debated. Moreover, there has been much debate about the need for repeat thyroid function tests for preterm infants (Cuestas et al., 1979 and Wilson et al., 1982) and thyroid hormone replacement in hypothyroxinemia of prematurity.

Our results showed significantly lower plasma concentrations of T4 and TSH in premature than in term babies, as already described in the literature, we too found a positive correlation between gestational age and plasma concentrations of thyroid hormones. This difference was only found when comparing group A (28-31 weeks) with the other groups studied (B&C) at the end of the first week (test1), but there were no significant differences in serum T4 and TSH values when groups B&C are compared with each other. This is in agreement with other studies which found a correlation with gestational age or birth weight only when comparing premature infants less than 28 weeks gestation to older infants (Hirano et al., 1982). Also this is in agreement with Fuse et al., 1990, who stated that, there was a wide variability in TSH values in premature infants compared with more mature infants, reflecting the immaturity of the hypothalamic-pit-thyroid negative feedback system.
Transient hypothyroxinemia is common in preterm infants and is more severe in infants born at low gestational age. Also preterm babies had a higher incidence of transient hypothyroidism and required accurate follow up and close monitoring of thyroid function. However, the incidence of persistent hypothyroidism among preterm infants does not differ from that among term newborns (Uhrmann et al., 1978). Some studies suggest that low serum concentrations of thyroid hormone in the early period of life are associated with poor developmental outcomes (Perlman, 2001 and Gressens et al., 2002). Thyroid hormone supplementation is frequently used for treatment of hypothyroxinemia, but there is no conclusive evidence that it is beneficial (Rapaport et al., 2001 and Ogilvy-Stuart, 2002). In this study about 48% of preterm neonates had transient hypothyroxinemia and about 6% had transient hypothyroidism as revealed on day 6-7 (test 1) and all of them attained normal values by day 28-35 (test 2). T4 levels increased in all infants who had hypothyroxinemia or hypothyroidism without receiving thyroxin supplementation. Similar results are reported by different workers (Cuestas, 1978; Uhrmann et al., 1981; Franklin et al., 1986 and Murphy et al., 2004), but Frank et al., 1996 recommended that infants with transient hypothyroidism should be treated as soon as the diagnosis is made. It is reported that replacement therapy showed a reduction of morbidity of these neonates (Schonberger et al., 1981). Some authors reported that, in preterm infants born at <28 week gestation, it usually takes more than one month for T4 levels to reach levels equal to those of term infants. It has been reported that thyroxin supplementation of infants born at <28 weeks gestation results in a better neurodevelopmental outcome at 2, 5 and 10 years of age. These findings suggest that thyroxin supplementation may be beneficial for preterm infants born at <28 weeks gestation who have hypothyroxinemia (Van Wassenaer et al., 1997).

Although hypothyroxinemia and hypothyroidism found in preterm newborns were transient and tend to disappear by next follow up, demonstrating recovery of thyroid function, it seems worthwhile enrolling these babies in follow up protocols to monitor or correct gland function especially in first year of life. Up till now the optimum screening protocol of congenital hypothyroidism for preterm neonates is not settled. In this study all infants with thyroid dysfunction in the first few days after birth (test 1) attains normal values after 4-5 weeks (test 2), also there were no cases with delayed TSH rise diagnosed. So we believe that routine second screening test may not be needed due to increased costs with a very low yield of cases and to feedback regulation at this age. In test 2 (i.e. after 4-5 weeks), there was no significant difference in plasma concentrations of thyroid hormones (T4 & TSH) between any of the gestational age groups studied when compared with each other.

In screening infants for congenital hypothyroidism, one such pattern is “delayed TSH rise”. Infants with delayed TSH rise typically have a low thyroxin level but a normal TSH value on the first newborn screening test (collected at 2-5 days of age), whereas subsequent testing (collected at 2-6 weeks of age) shows a persistently low thyroxin but now elevated TSH level (Hunter et al., 1998). In this study no infant with a normal TSH in test 1 was found to have elevated level in test 2 i.e. no delayed TSH rise. This is in agreement with Korada, 2008 who compared baseline readings of thyroid stimulating hormone (TSH) in 2238 preterm infants with second samples taken from 2039 infants. No infant with a normal TSH concentration on first sampling was found to have a reading of >10mU/L on second sampling. The author concluded that repeat sampling may not be required with a lower screening threshold of 6mU/L. Also Vincent et al., 2002 suggested that, for the detection of permanent primary congenital hypothyroidism in VLBW infants, a routine second screening specimen may not be needed. Their study needs to be confirmed in a much larger series of patients. However, Rastogi and La Fronchi, 2010 recommended adding a second new born screening test in targeted new born populations, which would include low birth weight or preterm babies, acutely ill term babies, babies with significant congenital anomalies, same sex twins and those with specific drug exposure (steroids, dopamine or iodine), similar conclusions were reached in guidelines published by the Clinical Laboratory and Standard Institute (Miller et al., 2009). Dorota et al., 2005 also reported that, repeated thyroid function test (5-6 weeks after birth) are necessary for preterm infants, even though they may initially show normal thyroid function to avoid false negative results and identifies infants who require diagnostic management for primary permanent or transient hypothyroidism and hyperthyrotropinemia (which might result in compensated hypothyroidism).

Conclusion:

Premature infants especially those born before 31 week gestations, had a high incidence of transient thyroid dysfuction (hypothyroxinemia and less commonly hypothyroidism). The changes observed in preterm babies tend to disappear with time, demonstrating recovery of thyroid function. However, it seems worthwhile enrolling these babies in follow up protocols to monitor or correct gland function especially in first year of life. Up till now the optimum screening protocol of congenital hypothyroidism for preterm neonates is not settled. In this study all infants with thyroid dysfunction in the first few days after birth (test 1) attains normal values after 4-5 weeks (test 2), also there were no cases with delayed TSH rise diagnosed. So we believe that routine second screening test may not be needed due to increased costs with a very low yield of cases and to
limit repeat screening only to selected newborn populations (including preterm especially those born before 31 week gestations and sick newborn) in whom the incidence of thyroid dysfunction (hypothyroxinemia and hypothyroidism) are high.

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