PP132 - Mutations in Thyroid Hormone Beta Receptor Gene Identified in Children with Clinical Resistance to Thyroid Hormones

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Introduction: Patients with resistance to thyroid hormones (RTH) show different clinical features. Several mutations have been identified in them. **Objective:** To describe patients followed up since 2006 with RTH suspicion evaluated for mutations in thyroid hormone beta receptor (*THRß)* gene.

Methods: Children were followed up in our Endocrinology Department. Patient 1:10-yr-old boy with elevated T3, T4 and free T4, normal TSH in routine thyroid testing requested for overweight. Patient 2:0.7-yr- old boy with Down syndrome and elevated T3, T4 and free T4, normal TSH. Patient 3:Boy with abnormal results on neonatal screening, with elevated T3, T4, free T4 and TSH. Patient 4:4.7–yr-old girl with elevated T3, T4 and free T4, normal TSH in routine thyroid testing requested for low weight. Patient 5: 1-yr-old boy with elevated T3, T4 and free T4, normal TSH in routine thyroid testing requested for low weight. Patient 6:Boy with congenital hypothyroidism diagnosed by screening with elevated T3, T4, free T4 and TSH. Clinical manifestations: Patients 1, 4 and 5 showed palpitations, tachycardia.Familial antecedents: Patient 3 has two brothers with similar RTH profile. Patient 4 had a sister who died at 3 months of age and mother with confirmed RTH. Patient 6 had an aunt with RTH profile. Thyroid ultrasound. All patients had normal gland size except patient 6 who had an hypoplastic gland. Patient 4 showed goiter at follow up. Treatment: Patient 1 received metimazol; patients 1,4 and 5 beta blockers and patient 6 levothyroxine. Molecular biology analysis: genomic DNA was isolated from blood cells and the exons 7-10 of the *THRß* gene, including the flanking intronic regions were amplified by PCR. DNA sequences from each amplified fragment were performed with the Taq polymerase-based chain terminator method and using the specific forward and reverse THRß primers. Results. Direct sequence analysis revealed a novel missense mutation in exon 10 in patient 3, c.1329G>T transvertion that results in a p.K443N substitution and two known missense mutations: c.1357C>A, p.P453T (Patient 1)in exon 10 and c.949G>A, p. A317T (Patient 4) in exon 9.

Conclusion:THRß gene mutations were found in half of the patients with RTH, including a new mutation. Although goiter is a common feature in RTH, only one patient presented it. These findings support the importance of searching THRß gene mutations in suspected individuals to achieve an adequate follow-up and treatment in patients with RHT.

Keywords: *Thyroid Resistance; THRß gene; Mutation*

Others

PP94 - Hematocrit Correction: An Innovative Tool to Reduce the Recall Rate in Newborn Screening of Endocrinophaties

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Introduction: Newborn screening in Argentina includes Congenital Hypothyroidism and Congenital Adrenal Hyperplasia among other conditions. Thyrotropin (TSH) and 17OH Progesterone (17OHP) are rutinarily measured as biochemical markers in dried blood spots (DBS). Although many variables are controlled, hematocrit remains as an uncontrollable parameter while cut-off values for newborn screening determinations are based on an estimated hematocrit value of 55%.

Objective: To design a method for the estimation of hematocrit in DBS and to study its influence on neonatal screening of endocrinopathies.

Method: We used a 3.2 mm punch on a plate with 200 uL sodium lauryl sulfate (1.7 g/l) to measure hemoglobin value by spectrophotometry (550 nm). A hemoglobin calibration curve and 3 controls were used in each run. To estimate hematocrit a 3.2 factor was used. The technique was validated comparing 114 hematocrit values from hemograms (SYSMEX XE2100) corresponding to the same date of DBS extraction. We analyzed 1124 DBS samples from 2 centers, premature and term neonates (gestational age 25-41 weeks), with or without hospitalization. The TSH and 17OHP determinations were made with MPBiomedicals or PerkinElmer (DELFIA) reagents. Samples that exceeded the cutoff value were corrected by hematocrit and then reclassified (recall or not recall).

Results: Linear regression between hematocrit values from DBS and hematocrit values from hemogram showed a Pearson coefficient: 0.957 and R2: 0.9143. From total analyzed samples 139 exceeded the cutoff value proposed by each laboratory: 49 for TSH and 90 for 17OHP. If hematocrit correction is used, only 57 (41.0%) patients would be recalled: 18 (out of 49) for TSH (36.7%) and 39 (out of 90) for 17OHP (43.3%). Mean hematocrit from 57 recalled samples was 52.5% (median: 52.3%) and from 82 reclassified as don't recall samples was 41.1% (median: 43.6%). From those samples which remain above the cutoff level (18 for TSH, 39 for 17OHP) after hematocrit correction, 3 (16.7%) for TSH and 19 (48.7%) for 17OHP were premature or low birth weight.

Conclusion: Hematocrit estimation would allow the correction of serum volume due to a smaller globular package in DBS samples with low hematocrit, which proved to be a determining variable in positive samples for TSH or 17OHP in DBS. The use of hematocrit estimation for samples that exceed cutoff value diminish the recall rate and avoid unnecessary anguish in families due to false positive results.

Keywords: *Newborn screening; Hematocrit; Endocrinophaties*