References values under Synacthen® test
for 6 steroids in serum by LC-MS/MS
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Background

The response to ACTH Test (Synacthen®) is a very useful for the screening of steroidogenesis enzymatic deficiency. With the development of steroid quantification by LC-MSMS more specific than most of immunoassays, the determination of reference value is required at basal and under stimulation time.

Objectives

Determination of references values for 6 steroids in serum by LC-MS/MS method and after Synacthen Test:
- 21-deoxycortisol (21DF),
- 11-deoxycortisol (11OH),
- Deoxycorticosterone (DOC),
- Corticosterone (Cortico),
- delta4 androstenedione (Delta4),
- 17-hydroxyprogesterone (17OHP)
using the same extraction and chromatography

Materials and Methods

Extraction of the Samples and calibration curve
-according to SLE method after addition of deuterium internal standard.

Steroid quantification: HPLC1290® Agilent Technology + mass spectrometer triple quadrupole 6460® Agilent technology. This method was validated according to the Norm (linear response, CV less than 10% for the repeatability, less than 15% for the reproducibility).

The limit of Quantification (nmol/L)

<table>
<thead>
<tr>
<th>Steroid</th>
<th>T0</th>
<th>T60</th>
</tr>
</thead>
<tbody>
<tr>
<td>21-DF</td>
<td>0.125</td>
<td>0.422</td>
</tr>
<tr>
<td>Cortico</td>
<td>0.125</td>
<td>0.522</td>
</tr>
<tr>
<td>11-OH</td>
<td>0.135</td>
<td>0.82</td>
</tr>
<tr>
<td>Delta 4</td>
<td>0.125</td>
<td>0.482</td>
</tr>
<tr>
<td>DOC</td>
<td>0.125</td>
<td>0.282</td>
</tr>
<tr>
<td>OHP</td>
<td>0.125</td>
<td>0.74</td>
</tr>
</tbody>
</table>

Reference values were performed for these 6 steroids after Synacthen® (T0, T60) in a cohort of patients previously studied in radioimmunoassay for 17OHP and 21DF and genetic status (normal, heterozygous, non-classical form) for mutation of CYP21A2 confirmed by sequencing.

In the normal group (normal response to Synacthen® determined in radioimmunoassay), steroids were quantified at T0 and T60 min (peak of stimulation for 17OHP and 21DF) for 55 patients.

Results

Normal values of 6 steroids after Synacthen® (T0, T60) for 55 patients

<table>
<thead>
<tr>
<th>Steroid</th>
<th>T0</th>
<th>T60</th>
</tr>
</thead>
<tbody>
<tr>
<td>21 DF</td>
<td>&lt;0.130</td>
<td>0.422</td>
</tr>
<tr>
<td>Cortico</td>
<td>6.833</td>
<td>52.82</td>
</tr>
<tr>
<td>11-OH</td>
<td>3.88-10.62</td>
<td>46.4-68.32</td>
</tr>
<tr>
<td>Delta 4</td>
<td>4.0-1.72</td>
<td>3.0-2.16</td>
</tr>
<tr>
<td>DOC</td>
<td>1.26-4.43</td>
<td>2.24-5.69</td>
</tr>
<tr>
<td>OHP</td>
<td>1.43</td>
<td>3.80</td>
</tr>
</tbody>
</table>

Chromatography method separated the followed steroids

21-DF Cortico 11-OH Delta 4 DOC OHP

T0 median
1-3* quartile (min-max)
6.833 (3.88-10.62)
2.79 (4.0-1.72)
0.125 (1.26-4.43)
0.125 (1.03-0.984)

T60 median
1-3* quartile (min-max)
52.82 (46.4-68.32)
3.80 (2.24-5.69)
0.125 (0.26-0.76)
0.125 (0.123-1.40)

After ACTH test, 21DF (<0.6nmol/L) exclude heterozygous for a mutation of the CYP21A2 gene. All non classical form for the 21-hydroxylase deficiency have a value of 17OHP under ACTH test superior to 40 nmol/L confirming our previous studies (Tardy et al. Hormon Research 2005;64,p41).

Conclusions

An evaluation of normal values for these 6 steroids may be useful to diagnose affected parents and prevent risk of Congenital Adrenal Hyperplasia for children. The utilization of LC-MS/MS method showed a reliable, sensitive and specific method to detect disorders of steroid biosynthesis when multiple precursors were high.