

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)

21-DF	Cortico	11-OH	Delta 4	DOC	OHP
0.125	0.125	0.135	0.125	0.125	0.125

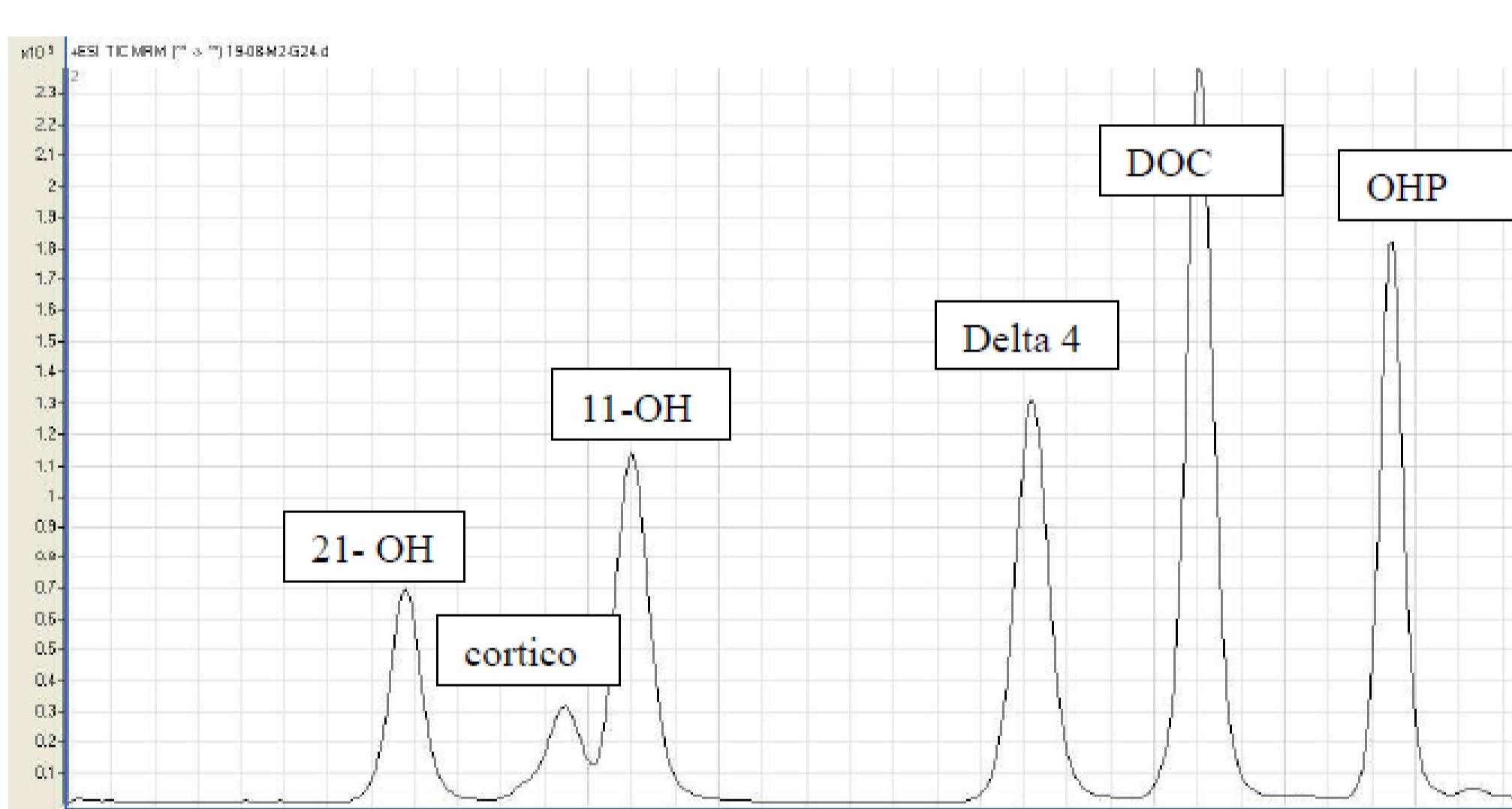
Reference values were performed for these 6 steroids after Synacthen® (T0, T60) in a cohort of patients previously studied in radioimmunoassay for 17OHP and 21DF and genetic status (normal, heterozygous, non- classical form) for mutation of CYP21A2 confirmed by sequencing.

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

	21 DF nmol/L	Cortico nmol/L	11-OH nmol/L	Delta 4 nmol/L	DOC nmol/L	OHP nmol/L
T0						
médiane	<0.130	6.833	0.6	2.79	0.125	1.43
1-3 ^e quartile (min-max)	3.88-10.62 (0.13-0.80)	46.4-68.32 (0.80-59.45)	0.4-1.72 (0.13-12.4)	1.26-4.43 (0.436-8.931)	0.13-0.14 (0.13-0.984)	0.87-2.79 (0.268-6.34)
T60						
médiane	0.422	52.82	2.84	3.80	0.374	3.74
1-3 ^e quartile (min-max)	0.32-0.84 (0.13-2.02)	16.2-97.3 (1.06-29.4)	3.02-4.16 (0.57-10.9)	2.24-5.69 (0.123-1.40)	0.26-0.76 (1.32-8.85)	3-6.46



Chromatography method separated the followed steroids

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.

