PLASMA STEROID PANEL WITH LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY (LC/MS-MS) METHOD: USAGE IN DIFFERANTIAL DIAGNOSIS OF HYPERANDROGENISM

Mert Uçar ¹, Aysun Ata², Burcu Barutçuoğlu³, Güneş Ak³, Sara Habif³, Zuhal Parıldar³, Damla Gökşen², Şükran Darcan², Samim Özen²

- 1. Ege University, Faculty of Medicine, Department of Pediatrics, İzmir, Turkey
- 2. Ege University, Faculty of Medicine, Division of Pediatric Endocrinology and Diabetes, İzmir, Turkey
- 3. Ege University, Faculty of Medicine, Department of Clinical Biochemistry, İzmir, Turkey



INTRODUCTION

- The etiology of hyperandrogenism in children and adolescent girls is often premature adrenarche (PA) before puberty, the leading cause in the adolescent period is polycystic ovary syndrome (PCOS). The final diagnosis is based on history, clinical findings, reliable measurement of steroid hormones and, if necessary, genetic analyzes.
- In the electrochemiluminescent immunoassay (ECLIA) method, the presence of various endogenous compounds (such as high rheumatoid factor) and the use of certain pharmaceutical agents (such as high-dose biotin) may cause interference in the results of androgens. Liquid chromatography-mass spectrometry (LC-MS / MS) method, which has become widespread in recent years, allows measurement of 17 adrenal steroids at the same time without these interferences.

 To evaluate the effectiveness of steroid hormone measurement by **LC-MS / MS** method and to compare the results with **ECLIA / ELISA** methods.

	DHEA	DHEA-S	DHEA-S	DHEA-S	17-OHPG	17-OHPG	17-OHPG	T.Testoste ron	T.Testoste ron	T.Testoste ron	DHT	Androste nedion
	LC- MS/MS (ng/mL)	LC- MS/MS (µg/dL)	ECLIA (μg/dL)	p	LC- MS/MS (ng/dL)	ELISA (ng/dL)	p	LC- MS/MS (ng/dL)	ECLIA (ng/dL)	p	LC- MS/MS (pg/mL)	LC- MS/MS (ng/dL)
PA n:37 (%38.5)	3.2 (0.7-458)	42.1 (0.1- 192.9)	118.9 (28.1- 375.7)	<0.001	38.2 (0.3- 355)	115 (0.3- 396.4)	<0.001	9.8 (2-58)	5.4 (0.1-34)	0.243	48.3 (0.1-928)	29.6 (6.3- 783.7)
PCOS n:24 (%25)	10.1 (1.6- 111.3)	179.9 (4.90- 434.4)	377 (77.7- 625)	<0.001	59 (0.2- 267.4)	263 (2-620)	<0.001	33.5 (12.7- 84.5)	50 (6-69)	0.488	93.1 (2-718.8)	170.3 (46- 442.3)
idiopatic hirsutism n:30 (%31.2)	3.6 (0.4- 157.8)	59.7 (0.7- 618.4)	118 (8.3-214)	0.001	24.1 (0.4- 131.8)	117 (0.8-220)	<0.001	9.6 (3.8-41.1)	3 (0-37)	0.01	64.4 (7.1- 976.6)	28.9 (1.5- 301.6)
Non-classic CAH n:5 (%5.2)	7.2 (4.3- 23.9)	51.1 (18- 134.1)	83.6 (54.6- 205.4)	0.225	467.5 (210- 1338)	601.4 (197.2- 2248)	0.07	10.2 (7-33)	13.4 (3-25.1)	0.06	37.4 (15.6- 586)	66.6 (22-177
Total n:96 (%100)	4.2 (0.4-458)	61.2 (0.14- 618.4)	124.7 (8.3-625)	<0.001	38.5 (0.2- 2248)	159 (0.3- 1338)	0.001	13.5 (2-84.5)	9.5 (0-69)	0.223	62.7 (0.1- 976.6)	44.3 (1.5- 783.7)

METHOD

Patients

- > 96 girls: 6-18 years old
- > PA, hirsutismus and PCOS diagnosis

Laboratory

- > Serum total testosterone and DHEA-S: ECLIA (Cobas e-801, Roche Diagnostics GmbH)
- > Plasma steroid hormones: total testosterone, DHEA, DHEA-S, DHT, androstenedione, 17-OH progesterone (17-OHPG): LC-MS / MS (6460 Triple Quadrupole LC / MS, Agilent Technologies, US)
- > Serum 17- OHPG: ELISA (Diametra, SnL, Italy)
- Normal values were evaluated according to Tanner stages

RESULTS

Patients

- > 37 PA (38.5%)
- > 5 non-classical CAH (5.2%)
- > 30 idiopathic hirsutismus (31.2%)
- > 24 PCOS (25%)
- menstrual cycles of was regular in 10(41.6%) PCOS cases
- secondary amenorrhea or oligomenorrhea in 14(58.4%)

Laboratory by LC-MS/MS

- ➤ DHEA个: 36 (37.1%)
- \rightarrow Androstenedione \uparrow : 18(18.6%)
- ➤ Dihydrotestosterone ↑: 20(20.8%)
- ➤ DHEA-S个: 15(15.8%)

- \rightarrow Total testosterone \uparrow : 6(6.4%)
- At least one androgen increase was detected in 58(60%) patients
- In 21 of cases (21.8%), 17-OHPG levels above 200ng/dL by ELISA



normal values were found in 16(76.1%) of them by LC-MS/MS

In 5(5.2%) cases, genetically proven non-classical CAH was diagnosed

CONCLUSIONS

- > Evaluating children and adolescent girls with hyperandrogenism; LC-MS/MS method is more valuable then other mehthods
- ➤ By LC-MS/MS, false positive 17-OHPG and DHEA-S measurements will be minimized and unnecessary investigations can be prevented
- > DHEA was the highest androgen/androgen precursor in patients with PA, PCOS and hirsutismus

