Measurement of serum 17-hydroxyprogesterone by LC-MS/MS is associated with a lower probability of false positive results compared to immunoassay as a screening for 21-hydroxylase-deficient nonclassic adrenal hyperplasia in woman with hyperandrogenism.

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Short title: 17-hydroxyprogesterone measured by LC-MS/MS

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Key words:

liquid chromatography/mass spectrometry, immunoassays, nonclassic congenital adrenal hyperplasia, hyperandrogenism

Abstract:

Basal serum 17OHP measurement remains the first screening step for NCCAH and the accuracy of the test is of high value.

The aim of this study was to compare the accuracy of immunoassays to LC-MS/MS in the assessment of serum 17OHP and androgens concentration in woman with hyperandrogenism and controls.

17OHP, total testosterone, androstendione and DHEAS were measured in 39 women with clinically and/or biochemically evident hyperandrogenism and in 29 age-matched controls without clinical hyperandrogenism. 17OHP and androgens were measured by immunoassays and by LC-MS/MS.

In patients group 17OHP level measured by immunoassays was significantly higher compared to LC-MS/MS (ELISA NovaTec® *vs* LC-MS/MS P<0.0001, ELISA DRG® *vs* LC-MS/MS P<0.0001) as well as in control group (ELISA DRG® *vs* LC-MS/MS P<0.0001). Additional, unnecessary diagnostic procedures explaining elevated 17OHP level were undertaken in 85% of patients when NovaTec® test was used, in 50% when ELISA DRG® and in none when LC-MS/MS method was applied. Total testosterone, androstendione and DHEAS concentrations in the patients and the controls assessed by the immunoassays were also significantly higher compared to LC-MS/MS.

LC-MS/MS is more reliable diagnostic tool in the measurement of serum 17OHP and androgens concentrations compared to immunoassays in woman with hyperandrogenism.

Introduction

Hyperandrogenism represents one of the most common endocrinopathy affecting about 10 % of woman of reproductive age and provides a negative impact on patients quality of life [1,2,3]. Nonclassic congenital adrenal hyperplasia (NCCAH) is specific, identifiable androgenic disorder, which needs to be confirmed by 17-hydroxyprogesterone (17OHP) measurement [4,5,6,7,8,9]. It is essential however to use reliable assays for quantifying 17OHP. The development of automated platforms gave direct assays the advantages of being convenient and relatively cheap, but on the other hand the assays occurred frequently to overestimate the measurements due to the lack of specificity of the antibodies. False positive 17OHP results may lead to patients distress and unnecessary, costly procedures. Therefore, there is a need of high validity methods in screening 17OHP measurements in order to select a group of patients for further diagnostic approach. In the past decade liquid chromatography coupled with mass spectrometry (LC-MS/MS) has revolutionized measurement of steroid hormones in various body fluids improving sensitivity, specificity and enabling automation of measurement [10,11].

The aim of the study was to compare the accuracy of widely used immunoassays with LC-MS/MS method in the assessment of serum 17OHP and androgens concentration in woman with hyperandrogenism and controls.

Material and Methods:

Subjects

39 women (18-45 year old, mean age 25.9) with clinically and/or biochemically evident hyperandrogenism were included into the study. All patients were hospitalized in the Department of Internal Medicine and Endocrinology, Medical University of Warsaw between June 2012 and February 2014.

The control group consisted of 29 healthy woman aged 19-45years (mean age 27.9) with regular menses, without clinical hyperandrogenism (acne and/or androgenic alopecia and/or hirsutism and/or virilization). Exclusion criteria for both groups were as follows: age < 18 years or > 45 years, pregnancy, recent history (until 3 months) of hormonal contraceptive or hormonal replacement therapy use, thyroid dysfunction, hyperprolactinemia.

All the patients undergone clinical and hormonal assessment as well as pelvic and adrenal ultrasound (US). In case of adrenal tumor suspicion on US, computed tomography of adrenal glands was performed.

Clinical evaluation of the patients was based on anthropometric measurements (weight, height), body mass index (BMI), age, the data of menstruation regularity, presence of hirsutism, acne, androgenic *alopecia*, virilization, presence of cushingoid features. Menstrual cycles shorter than 25 and longer than 34 days were considered abnormal. Hirsutism was defined by a Ferriman-Gallwey score ≥ 8 [12], presence of acne and *alopecia* was recorded but not scored.

Biochemical measurements

Laboratory tests consisted of serum total testosterone (TT), dehydroepiandrostrone sulphate (DHEA-S), androstendione, 17OHP, sex hormone binding globulin (SHBG), albumin, TSH, free T4, prolactin, glucose and insulin measurements. Biochemical hyperandrogenism was defined as serum total testosterone, DHEA-S or androstendione above upper range of normal

values obtained by immunoassays. In case of clinical or biochemical suspicion of Cushing's syndrome, NCCAH or CAH further diagnostic procedures were undertaken. The decisions about further diagnosis were based on results obtained by immunoassays.

Cushing's syndrome was diagnosed according to Endocrine Society guidelines [13].

In case of basal serum 17OHP > 5.1-30 nmol/l according to Escobar-Morreale et al. [14] ACTH stimulation test (17OHP measurement 30' and 60' post ACTH *im.* injection) or urine steroid profile was performed. When basal or post-stimulated 17OHP ≥ 30 nmol/l *CYP21A2* genetic analysis and/or urine steroid profile was performed [6].

The blood samples for hormonal assessment were collected in the morning (7.00-9.00 am) during the early follicular phase of the menstrual cycle (3^{th}-5^{th} day).

Assessment of TT, DHEA-S, SHBG and albumin concentrations in patients group was performed using ECLIA Cobas® diagnostic kits (Roche Diagnostics GmbH, Mannheim, Germany). 17OHP measurements were performed using ELISA NovaTec® (NovaTec Immunodiagnostica GmbH, Dietzenbach, Germany), androstendione using ELISA DRG® (DRG Instruments GmbH, Marburg, Germany) kits in the commercial laboratory. Independently, individuals from the studied groups had assessed serum TT, androstendione, 17OHP levels by the ELISA DRG® (DRG Instruments GmbH, Marburg, Germany) and DHEA-S by ECLIA Elecsys® (Roche Diagnostics GmbH, Mannheim, Germany) kits assessed according to the manufacturer's protocol. In all immunoassays intra- and interassay variability did not exceed 10 %.

Free testosterone was calculated with the use of the formula available at http://www.issam.ch/freetesto.htm.

TT, DHEA-S, androstendione and 17OHP were also assessed by LC-MS/MS in both groups.

LC-MS/MS measurements were performed in the Mass Spectrometry Laboratory of the Institute of Biochemistry and Biophysics, Polish Academy of Sciences, samples were analyzed using AbsoluteIDQ Stero17 Kit produced by Biocrates Life Sciences AG (Innsbruck, Austria) according to the protocol supplied by the manufacturer. The intra- and interassay variability did not exceed 11.5%.

TSH, free T4, prolactin and cortisol were measured using ECLIA Cobas® diagnostic kits (Roche Diagnostics Limited, Burgess Hill, UK).

Urine steroid profile was performed by capillary gas chromatography/mass spectrometry in selective ion monitoring mode (GC/MS-SIM) in the Department of Biochemistry, Radioimmunology and Experimental Medicine, Children's Memorial Health Institute, Warsaw, Poland [15].

Genetic testing was performed commercially in Genomed Health Care Center (Warsaw, Poland). Total genomic DNA was isolated from peripheral blood leukocytes according to Miller et al.[16]. The *CYP21A2* gene was analyzed by direct sequencing.

Differential diagnosis

Polycystic ovary syndrome was defined according to Rotterdam criteria [17].

NCCAH/CAH was diagnosed in patients with clinical hyperandrogenism and basal and/or post stimulated 17OHP ≥ 30 nmol/l when confirmed by *CYP21A2* genetic analysis and/or urine steroid profile [6].

Idiopathic hyperandrogenism was diagnosed in case of presence of clinical and biochemical hyperandrogenism, regular menses and normal ovaries on ultrasound [18].

The project has been approved by the Bioethical Committee of the Medical University of Warsaw, and a written informed consent for participation in this study was obtained from all participants. The basic clinical characteristics of the studied individuals as well as of the control group are summarized in Table I.

Statistical analysis

The differences in 17OHP and androgens concentrations were assessed with Statistica software package v.10 (StatSoft, Tulsa, OK), using the Student's t/Mann-Whitney U test. All correlations between quantitative values were performed with the Spearman correlation test. To compare the number of the individuals with the results of 17OHP > 5.1 nmol/l obtained with the different methods a Chi-square test with a 2x2 or 3x2 contingency tables was used. For all tests, the level of significance was established at 0.05.

Results

Comparison of the different diagnostic methods in the assessment of serum 17-hydroxyprogesterone and androgens concentrations.

17- hydroxyprogesterone

The results of serum 17OHP concentrations measured by the three different diagnostic methods are summarized in Table II. 17OHP levels obtained by both immunoassays were significantly higher than by LC-MS/MS (P<0.0001). There was also significant difference between 17OHP level assessed by ELISA NovaTec® *vs* ELISA DRG® (P=0.006) (Figure 1). Even though there was a positive correlation between the measurements obtained by ELISA NovaTec® and by LC-MS/MS (r=0.98, P<0.0001), ELISA DRG® and LC-MS/MS (r=0.99, P<0.0001) and ELISA NovaTec® and ELISA DRG® (r=0.98, P<0.0001). The use of the different methods was associated with a different probability of obtainment of 17OHP results requiring further diagnosis towards NCCAH. 20 out of 36 of the patients (55.5%) had elevated 17OHP levels when measured by ELISA NovaTec®. When ELISA DRG® kit was used 6 out of 31 (19.4%) had 17OHP concentrations above 5.1 nmol/l but measured by LC-MS/MS only 3 out of 38 (7.9%) individuals. There was a significant difference in the percentage of the patients with elevated 17OHP measured by ELISA NovaTec® comparing to LC-MS/MS as well as ELISA DRG® *vs* LC-MS/MS and ELISA NovaTec® *vs* ELISA DRG® (P<0.0001).

In the control group the median concentration of 17OHP assessed by ELISA DRG® was significantly higher compared to that obtained by LC-MS/MS (P<0.0001). There was significant difference in median 17OHP concentration between patients and controls when assessed either by ELISA DRG® or LC-MS/MS (P=0.0009 and P=0.0026 respectively) (Figure 1).

In the control group when ELISA DRG® was used 1 patient out of 29 (4.4%) had 17OHP level elevated. None of the healthy individuals had 17OHP > 5.1 nmol/l measured by LC-MS/MS. Measurement of 17OHP concentrations by ELISA NovaTec® was not performed in the control group.

Androgens (total testosterone, androstendione, DHEA-S)

The results of serum androgens concentrations measurements by the immunoassays and LC-MS/MS are summarized in Table II.

Evaluation of patients with elevated 17OHP serum level/final diagnosis

The decision towards further diagnostics were undertaken based on the results obtained from ELISA NovaTec® test. In all cases, however, when 17OHP was > 5.1 nmol/l in ELISA DRG® it was also elevated in ELISA NovaTec® and when was elevated in LC-MS/MS it was also elevated in both immunoassays.

In all, but one patient in case of basal 17OHP level 5.1-30 nmol/l further diagnostics has been done. One patient presented with clinical picture of Cushing's syndrome and finally was diagnosed with adrenal cortical carcinoma (ACC). ACTH stimulation test was performed in 14 patients, urine steroid profile in 7 and genetic testing in 3. 17OHP exceeded 30 nmol/l post ACTH stimulation in 2 patients, but further evaluation didn't confirm NCCAH. Urine steroid profile was positive in 2 patients with the diagnosis of NCCAH and CAH. Genetic testing towards *CYP21A2* mutations was negative in all 3 cases in whom was performed.

Final diagnosis revealed in 24 (61.5%) of the patients polycystic ovary syndrome (PCOS),12 (31%) idiopathic hyperandrogenism, 2 (5%) patients were diagnosed with CAH (1 with classic and 1 with nonclassic form based on urine steroid profile) and 1 (2.5%) with ACC.

Characteristic of the immunoassays and LC-MS/MS based on 17OHP serum measurements

ELISA NovaTec®

Sensitivity 100%, specificity 50%, false positive results 50%, false negative results 0%, positive predictive value 15%, negative predictive value 100%, test accuracy 54%.

ELISA DRG®

Sensitivity 100%, specificity 92.98%, false positive results 7%, false negative results 0%, positive predictive value 42.86%, negative predictive value 100%, test accuracy 93.33%.

LC-MS/MS

Sensitivity 100%, specificity 100%, false positive results 0%, false negative results 0%, positive predictive value 100%, negative predictive value 100%, test accuracy 100%.

Discussion

Our study showed excellent accuracy of LC-MS/MS method in 17OHP measurement compared to immunoassays in screening for 21-hydroxylase-deficient nonclassic adrenal hyperplasia in woman with hyperandrogenism.

In the study 17OHP levels measured by LC-MS/MS were significantly lower in patients and in the control group compared to both immunoassays and confirm the results obtained by Fanelli et al. and Koal et al. [19,20]. Our results showed also that use of immunoassays is associated with a higher probability of false-positive results in women with hyperandrogenism especially when measured by ELISA NovaTec® kit. Common use of immunoassays may therefore introduce further unnecessary diagnostic procedures, needless hospitalizations, additional stress for the patients and finally – lead to the false diagnosis. Our results indicate however that negative 17OHP level obtained by validated immunoassays speaks against NCCAH. Unnecessary and costly diagnostic procedures were undertaken in 17/20 (85%) patients when the NovaTec® test was used and in 3/6 (50%) when ELISA DRG® and in none when LC-MS/MS method was applied. Results of this study are consistent with previous reports regarding the comparison of immunoassays and LC-MS/MS for 17OHP measurements in screening for CAH during the neonatal period [6, 21, 22, 23]. However one should remember that the proposed basal 17OHP cut off level speaking for or against NCCAH was established based on immunoassays [9,14]. 17OHP cut off level by LC-MS/MS remains to be established, otherwise use of this method may lead to the underdiagnose of NCCAH, although it was not seen in case of our patients.

According to the literature, major benefits of LC-MS/MS, apart from the high sensitivity and specificity, include also small sample size required for an assay, short term of sample preparation and possibility of measurement of multiple steroids simultaneously [20,24,25]. The only disadvantage is limited access to this method as well as the cost of a single

parameter. In our study, apart from the 17OHP measurements, we also compared immunoassays with LC-MS/MS results in the assessment of androgens concentrations.

In the patients group as well as in the controls we have observed significantly lower levels of total testosterone, androstendione and DHEA-S when measured by LC-MS/MS compared to immunoassays. Similar discrepancies between HPLC-MS/MS and immunoassay based analysis for androstenedione were obtained by Fanelli et al. confirming the limitation of tested immunoassays due to the lack of immunoassay specificity by cross-reactivities with matrix components resulting in overestimation of the measurements [19]. These findings support previous studies showing that LC-MS/MS is characterized by a good precision and high accuracy in the measurement of androgens in women, compared with the immunoassays [20,26,27].

Our results support the data of other investigators that in measurement of steroids, especially for high quality clinical research, LC-MS/MS – based assays are the method of choice, so introduction of this method to commercial laboratories is probably a question of time [28].

Declaration of Interests

The authors report no conflicts of interest.

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References:

1. Carmina E, Rosato F, Janni A, Rizzo M, Longo RA. Relative prevalence of different androgen excess disorders in 950 women referred because of clinical hyperandrogenism. J Clin Endocrinol Metab 2006;91:2-6.
2. Yildiz BO. Diagnosis of hyperandrogenism: clinical criteria. Best Pract Res Clin Endocrinol Metab 2006;20:167-176.
3. Ehrmann DA. Polycystic ovary syndrome. N Engl J Med 2005;352:1223-1236.
4. Azziz R, Sanchez A, Knochenhauer ES, Moran C, Lazenby J, Stephens KC, Taylor K, Boots LR. Androgen excess in women: experience with over 1000 consecuitive patients. J Clin Endocrinol Metab 2004;89:453-462.
5. AACE Hyperandrogenism Guidelines. Endocr Pract 2001;7:120-133.
6. Speiser PW, Azziz R, Baskin LS, Ghizzoni L, Hensle TW, Merke DP, Meyer-Bahlburg HF, Miller WL, Montori VM, Oberfield SE, Ritzen M, White PC. Congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency: an Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab 2010;95:4133-4160.
7. Ambroziak U, Bednarczuk T, Ginalska-Malinowska M, Małunowicz EM, Grzechocińska B, Kamiński P, Bablok L, Przedlacki J, Bar-Andziak E. Congenital adrenal hyperplasia due to 21-hydroxylase deficiency-management in adults. Endokrynol Pol 2010;61:142-155.
8. New MI. Nonclassical 21-hydroxylase deficiency. J Clin Endocrinol Metab 2006;91:4205-4214.

9. Azziz R, Hincapie LA, Knochenhauer ES, Dewailly D, Fox L, Boots LR. Screening for 21-hydroxylase-deficient nonclassic adrenal hyperplasia among hyperandrogenic women: a prospective study. Fertil Steril 1999;72:915-925.
10. Stanczyk FZ, Clarke NJ. Advantages and challenges of mass spectrometry assays for steroid hormones. J Steroid Biochem Mol Biol 2010;121:491-495.
11. Honour JW. 17-hydroxyprogesterone in children, adolescents and adults. Ann Clin Biochem 2014;51:424-440.
12. Ferriman D, Gallwey JD. Clinical assessment of body hair growth in women. J Clin Endocrinol Metab 1961;21:1440-1447.
13. Nieman LK, Biller BM, Findling JW, Newell-Price J, Savage MO, Stewart PM, Montori VM. The diagnosis of Cushing's syndrome: an Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab 2008;93:1526-1540.
14. Escobar-Morreale HF, Sanchón R, San Millán JL. A prospective study of the prevalence of nonclassical congenital adrenal hyperplasia among women presenting with hyperandrogenic symptoms and signs. J Clin Endocrinol Metab 2008;93:527-533.
15. Małunowicz EM, Mitkowska Z, Bal K, Nizankowska-Błaz T, Moszczyńska E, Iwanicka Z, Romer TE. Definitive diagnosis of enzymatic deficiencies of steroidogenesis in at-risk newborns and infants by urinary marker analysis using GC-MS/SIM. Horm Res 1997;48:243-251.
16. Miller SA, Dyke DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16:1215.
17. Rotterdam ESHRE/ASRM Sponsored PCOS Consensus Workshop Group 2004. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod 2004;19:41-47.
18. Carmina E, Longo RA, Rini GB, Lobo RA. Phenotypic variation in hyperandrogenic women influences the finding of abnormal metabolic and cardiovascular risk parameters. J Clin Endocrinol Metab 2005;90:2545-2549.
19. Fanelli F, Belluomo I, Di Lallo VD, Cuomo G, De Iasio R, Baccini M, Casadio E, Casetta B, Vicennati V, Gambineri A, Grossi G, Pasquali R, Pagotto U. Serum steroid profiling by isotopic dilution-liquid chromatography-mass spectrometry: comparison

with current immunoassays and reference intervals in healthy adults. Steroids 201;76:244-253.

20. Koal T, Schmiederer D, Pham-Tuan H, Röhring C, Rauh M. Standardized LC–MS/MS based steroid hormone profile-analysis. J Steroid Biochem Mol Biol 2012;129:129-138.
21. Soldin SJ, Soldin OP. Steroid hormone analysis by tandem mass spectrometry. Clin Chem 2009;55:1061-1066.
22. Janzen N, Peter M, Sander S, Steuerwald U, Terhardt M, Holtkamp U, Sander J. Newborn screening for congenital adrenal hyperplasia: additional steroid profile using liquid chromatography-tandem mass spectrometry. J Clin Endocrinol Metab 2007;92:2581-2589.
23. Rossi C, Calton L, Hammond G, Brown HA, Wallace AM, Sacchetta P, Morris M. Serum steroid profiling for congenital adrenal hyperplasia using liquid chromatography-tandem mass spectrometry. Clin Chim Acta 2010;411:222-228.
24. Speiser PW. Improving neonatal screening for congenital adrenal hyperplasia. J Clin Endocrinol Metab 2004;89:3685-3686.
25. Etter ML, Eichhorst J, Lehotay DC. Clinical determination of 17-hydroxyprogesterone in serum by LC-MS/MS: comparison to Coat-A-Count RIA method. J Chromatogr B Analyt Technol Biomed Life Sci 2006;840:69-74.
26. Janse F, Eijkemans MJ, Goverde AJ, Lentjes EG, Hoek A, Lambalk CB, Hickey TE, Fauser BC, Norman RJ. Assessment of androgen concentration in women: liquid chromatography-tandem mass spectrometry and extraction RIA show comparable results. Eur J Endocrinol 2011;165:925-933.
27. Keefe CC, Goldman MM, Zhang K, Clarke N, Reitz RE, Welt CK. Simultaneous measurement of thirteen steroid hormones in women with polycystic ovary syndrome and control women using liquid chromatography-tandem mass spectrometry. PLoS One 2014;9:93805.
28. Handelsman DJ, Wartofsky L. Requirement for mass spectrometry sex steroid assays in the Journal of Clinical Endocrinology and Metabolism. J Clin Endocrinol Metab 2013;98:3971-3973.

Table I. Clinical characteristics of the studied groups.

	Patients with HA (N=39)	Controls (N=29)	P value
Age, mean (years, min-max)	25.9 (18-45)	27.9 (19-45)	0.52
BMI, mean (min-max)	25.5 (17.7-47.6)	23.3 (18.6-46.8)	0.36
Waist circumference, mean (cm, min-max)	78.5 (61-112)	76.2 (63-113)	0.80
% of adipose tissue* mean, (min-max)	32.9 (21.2-50)	29.1 (15.8-41.6)	0.11
Ferriman-Gallwey score, mean (min-max)	5.28 (0-18)	0.59 (0-7)	<0.0001
Acne, n (%)	25 (64.1)	0	<0.0001
Alopecia, n (%)	7 (17.9)	0	<0.0001
Menstrual disturbances, n (%)	27 (69.2)	0	<0.0001
Insulin resistance, n (%)**	10 (25.6)	1 (3.3)	0.013
Diagnosis of PCOS, n (%)	24 (61,5)	-	
Diagnosis of IHA, n (%)	12 (31)	-	
Diagnosis of CAH, n (%)	2 (5)	-	
Diagnosis of ACC, n (%)	1 (2.5)	-	

HA – hyperandrogenism

PCOS – polycystic ovary syndrome

IHA – idiopathic hyperandrogenism

CAH – congenital adrenal hyperplasia

ACC – adrenal cortical carcinoma

n – number

*measured by the bioelectrical impedance method

**definied as HOMA index value ≥ 3.0

Table II. Comparison of 17OHP [nmol/l], TT [nmol/l], androstendione [nmol/l] and DHEA-S [µmol/l] concentrations assessed by different methods in women with hyperandrogenism and in control group.

	Patients with HA (median, min-max)	**Controls** (median, min-max)	**P value** (HA *vs* controls)
17OHP ELISA NovaTec® [N: < 5.1]	5.49 (2.31-2046)*	ND	
17OHP ELISA DRG® [N: < 5.1]	3.57 (1.11-639) **	2.58 (0.63-2.73)	0.0009
17OHP LC-MS/MS [N: < 5.1]	1.56 (0.45-756) ***	1.14 (1.08-4.56) ****	0.0026
TT ECLIA Cobas® [N: 0.29-1.67]	1.71 (0.8-44.16)#	ND	
TT ELISA DRG® [N: < 1.7]	1.8 (0.84-79.8) ##	1.29 (0.31-2.30)	0.0006
TT LC-MS/MS [N: 0.1-1.6]	1.28 (0.67-45.73) ###	0.91 (0.44-1.45) ####	0.0004
fT ECLIA Cobas®	0.023 (0.01-2.76)•	ND	
fT ELISA DRG®	0.025 (0.009-3.11)••	ND	
fT LC-MS/MS	0.018 (0.008-1.43) •••	ND	

androstendione ELISA DRG® commercial laboratory [N: 2.62-13.58]	16.05 (5.86-147.6)$^{\Delta}$	ND	
androstendione ELISA DRG® [N: < 13.58]	15.18 (0.76-122.64) $^{\Delta\Delta}$	12.73 (4.05-28.02)	0.202
androstendione LC-MS/MS [N: 1.22 -8.72]	6.49 (3.14-124.59) $^{\Delta\Delta\Delta}$	4.64 (0.44-2.91) $^{\Delta\Delta\Delta\Delta}$	0.002
DHEA-S ECLIA Cobas® [N: 4.03-11.07 age 20-24 2.68-9.25 age 25-34 1.66-9.17 age 35-44]	9.13 (2.92-27.2)§	ND	
DHEA-S ECLIA Elecsys® [N: 4.03-11.07 age 20-24 2.68-9.25 age 25-34 1.66-9.17 age 35-44]	9.43 (4.52-16.64) §§	7.75 (1.89-16.72)	0.228
DHEA-S LC-MS/MS [N: 1.09-8.70 age 18-29 1.09-8.84 age 30-49]	7.45 (1.39-122.36) §§§	6.68 (1.32-13.95) §§§§	0.193

HA – hyperandrogenism
N – normal values range
ND – not done
LC-MS/MS – liquid chromatography coupled with mass spectrometry
fT – free testosterone

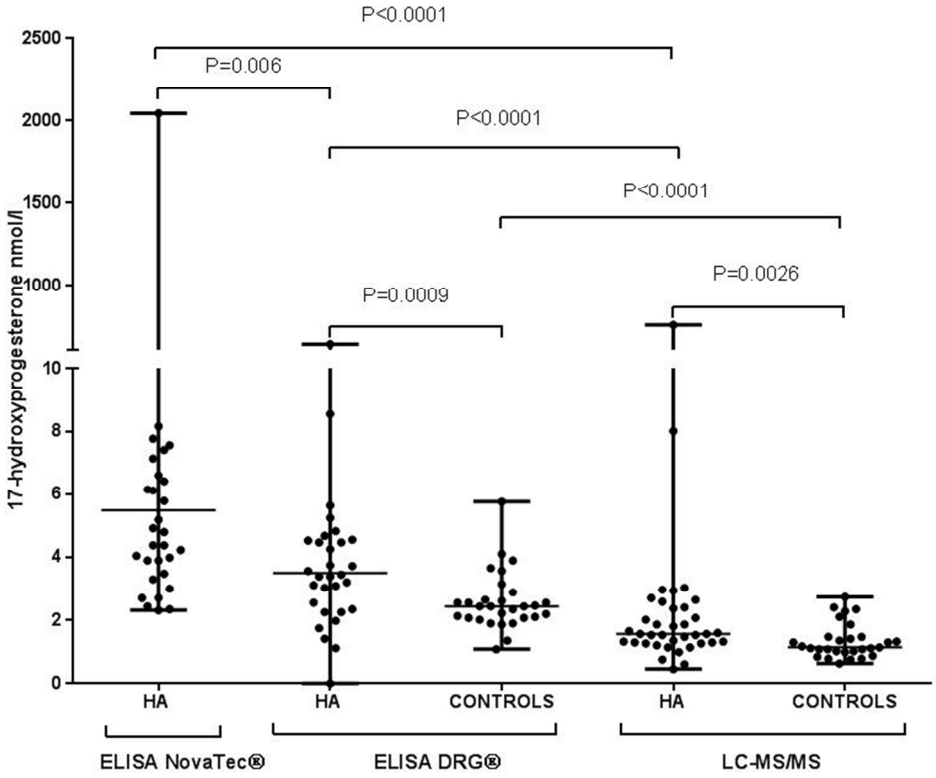
* Patients with HA: 17OHP ELISA NovaTec® *vs* 17OHP ELISA DRG® P=0.006
** Patients with HA: 17OHP ELISA NovaTec® *vs* 17OHP LC-MS/MS P<0.0001
*** Patients with HA: 17OHP ELISA DRG® *vs* 17OHP LC-MS/MS P<0.0001
**** Controls: 17OHP ELISA DRG® *vs* 17OHP LC-MS/MS P<0.0001

Patients with HA: TT ECLIA Cobas® *vs* TT ELISA DRG® P=0.593
Patients with HA: TT ECLIA Cobas® *vs* TT LC-MS/MS P=0.013
Patients with HA: TT ELISA DRG® *vs* TT LC-MS/MS P=0.009
Controls: TT ELISA DRG® *vs* TT LC-MS/MS P=0.002

• Patients with HA: fT ECLIA Cobas® *vs* fT ELISA DRG® P=0.806
•• Patients with HA: fT ECLIA Cobas® *vs* fT LC- MS/MS P=0.007
••• Patients with HA: TT ELISA DRG® *vs* fT LC-MS/MS P=0.02

Δ Patients with HA: androstendione commercial ELISA DRG® *vs* androstendione ELISA DRG® P=0.69
ΔΔ Patients with HA: androstendione commercial ELISA DRG® *vs* androstendione LC-MS/MS P<0.0001
ΔΔΔ Patients with HA: androstendione ELISA DRG® *vs* androstendione LC-MS/MS P<0.0001
ΔΔΔΔ Controls: androstendione ELISA DRG® *vs* androstendione LC-MS/MS P<0.00001

§ Patients with HA: DHEA-S ECLIA Cobas® *vs* DHEA-S RIA Elecsys® P=0.973
§§ Patients with HA: DHEA-S ECLIA Cobas® *vs* DHEA-S LC-MS/MS P=0.006
§§§ Patients with HA: DHEA-S ECLIA Elecsys® *vs* DHEA-S LC-MS/MS P=0.02
§§§§ Controls: DHEA-S ECLIA Elecsys® *vs* DHEA-S LC-MS/MS P=0.03



215x173mm (96 x 96 DPI)

Figure 1. Comparison of the 17OHP levels assessed by the different methods in the studied group of women with hyperandrogenism (HA) and in the control group.