Austin Journal of Invitro Fertilization

Open Access

Austin Publishing Group

Research Article

Androgen Profiling in Various Types of PCOS by Chemiluminescent Immunoassay in Combination with LC-MS

Xiaoyan D[1]*, Yanqin D[2], Yajun H[1], Qing X[1], Li L[1] and Chunlin W[1]

[1]Department of Gynecology and Obstetrics, The First Hospital of Wuhan, China

[2]Wuhan Agricultural Inspection Center, China

***Corresponding author:** Deng Xiaoyan, Department of Gynecology and Obstetrics, The First Hospital of Wuhan, No. 215 Zhongshan Avenue, Wuhan, China

Received: August 11, 2017; **Accepted:** September 18, 2017; **Published:** October 02, 2017

Abstract

149 Chinese PCOS patients with ovulation disorder and polycystic ovaries were recruited according to their serum T and DHEAS levels determined by CI and their F-G hirsutism score. The patients were divided into 2 subgroups: group A comprised 55 hyperandrogenism patients and group B included 94 patients without hyperandrogenism. The control group contained 82 patients with tubal infertility. We analyzed multiple androgens levels of all 231 patients by both chemiluminescent immunoassay and Liquid Chromatography/Mass Spectrometry (LC-MS) methods. Then the different methods and indexes of the hyperandrogenism for diagnosis and classification were compared. The study revealed that the LC-MS based method showed agreement with CI on the diagnosis of hyperandrogenism in PCOS patients. Although T, AD, DHEAS, and FTI levels were all specific parameters for the diagnosis of hyperandrogenism in PCOS, the T level and FTI value were more important factors for sub typing of Chinese PCOS types (hyperandrogenic or non-hyperandrogenic).

Keywords: PCOS; Sub typing; LC-MS; Hyperandrogenism

Introduction

Polycystic Ovary Syndrome (PCOS) is a common endocrine disorder characterized by hyperandrogenism and anovulatory infertility [1]. Hyperandrogenism has been considered as an important endocrine marker and is involved in the disease mechanism of PCOS, which is characterized by a high level of testosterone in serum [2,3]. According to the National Institutes of Health (NIH), the Rotterdam European Society for Human Reproduction/American Society of Reproductive Medicine and the Androgen Excess Society criteria, hyperandrogenism and other clinical symptoms have been used to establish a diagnosis of PCOS [2,4,5]. The specific biochemical characteristics of hyperandrogenism include elevated levels of Testosterone (T), Free Testosterone Index (FTI), and Androstenedione (AD), and an increase in endogenous adrenal hormones such as DHEAS is seen in 20–26% of PCOS patients [6,7]. In our study, both chemiluminescent immunoassay and liquid chromatography/mass spectrometry (LC-MS) methods were employed and compared for analysis of multiple androgens in PCOS patients. On the other hand, the goal of this study is to determine the value of different methods and different indexes of the hyperandrogenism for diagnosis and classification of PCOS patients in China.

Materials and Methods

Sample Selection

A total of 149 patients with ovulation disorder and PCOS were identified following the Rotterdam diagnostic criteria published in 2003(Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2004). The patients were divided into 2 groups based on their clinical symptoms (hirsutism) or biochemical characteristics of elevated androgens (T or DHEAS): group A comprised 55 patients with either hirsutism or hyperandrogenism with ovulation disorder and polycystic ovaries based on ultrasound evaluation, and group B comprised 94 patients with no clinical symptoms or biochemical characteristics of hyperandrogenism, but with symptoms of oligo-ovulation or anovulation and polycystic ovaries. Hirsutism was determined by a Ferriman-Gallwey (F-G) score ≥ 5 [8-10]. Hyperandrogenism was diagnosed based on the results of chemiluminescent immunoassays showing a T level ≥ 0.76 ng/mL [11] and/or with DHEAS levels above reference values for their corresponding age groups [12,13]. The control group was composed of 82 patients below age 40 with tubal infertility without menstrual disorders and hyperandrogenism. All the patients were tested for blood lipids, glucose, fasting insulin level, Body Mass Index (BMI), and basic hormones. All patients in the study had not received hormonal medicines within 3 months prior to the study.

Ethical considerations

All women gave their informed consent, and the study protocol was approved by the Medical Ethics Committee of the First Hospital of Wuhan.

Exclusion criteria

1. Any PCOS patients other than the above description.
2. Patients with other diseases that cause elevations in androgens or anovulation such as 21-hydroxylase deficiency, Cushing's syndrome, thyroid dysfunction, endocrine cancer, or premature ovarian failure.
3. Patients above 40 years old.
4. Patients with FSH > 25 IU/L.

Xiaoyan et al. © All rights reserved

Citation: Xiaoyan D, Yanqin D, Yajun H, Qing X, Li L and Chunlin W. Androgen Profiling in Various Types of PCOS by Chemiluminescent Immunoassay in Combination with LC-MS. Austin J In Vitro Fertili. 2017; 4(2): 1036.

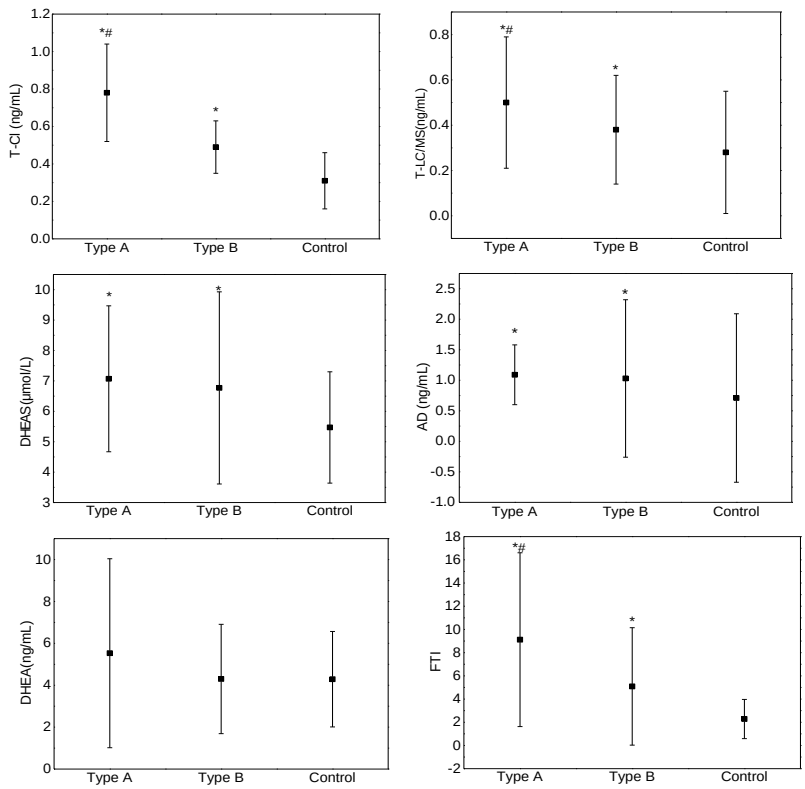

Figure 1: Androgen levels of the PCOS patient groups and controls.
Notes: *Compared to the control group, P<0.05; #compared to the B group, P<0.05.

5. Patients with prolactin (PRL) > 24 μg/L.

6. Patients with Thyroid-Stimulating Hormone (TSH) > 5.5 mIU/L.

7. Patients with other acute or chronic diseases.

8. For amenorrheic patients, blood β-HCG and progesterone were tested to exclude those who were pregnant or ovulating pregnant or ovulating.

All serum samples from the patient groups or the control group were collected during their second to fourth days of menstruation, or at any time for patients with amenorrhea. The samples were analyzed for Luteinizing Hormone (LH), FSH, T, Estradiol (E2), PRL, DHEAS, and SHBG using chemiluminescent immunoassay. The assay was performed on a Siemens ADVIA centaur XP automated chemiluminescent immunoassay system following the manufacturer's instructions. The reagents, calibrators and Quality Control (QC) materials were from the package supplied with the system and were used as suggested by the manufacturer. The rest of each serum sample was kept frozen at −80°C before testing using LC-MS for the levels of T, DHEA, and AD. The calibrators for T, DHEA, AD and the isotope-labeled internal standard DHEA-D6 were purchased from Sigma. The isotope-labeled internal standards T-13C3 and AD-13C3 were purchased from Cambridge Isotope Laboratories (CIL). Double charcoal-stripped serum was purchased from Sera Care. The mobile phase reagents were purchased from either Fisher or Sigma.

LC-MS parameters

The LC-MS was performed with a Shimadzu LC-30 AT liquid chromatography for sample separation with a mobile phase A of water, mobile phase B of methanol, and a flow rate of 0.4 mL/min. The LC method was a total of 9 min, starting with 50% B at 0.01 min, increased to 80% B for 8.00 min, and back to 50% B from 8.01 min, and terminated by 9 min. Sample injection volume was 50 μL. An AB Sciex Qtrap 4500 was used for mass spectrometry analysis with an APCI source and collision gas. Other key parameters were a curtain gas of 30 psi, spray current of 5.0μA, spray temperature at 400°C, and ion source Gas1 of 40 psi. According to a previously published method [14-18], the hormone molecules were measured using a Multiple Reaction Monitoring (MRM) technique. The mass transitions used (Q1/Q3, m/z) for each target molecule and internal standards were as follows, DHEA 271.2/213.2, DHEA-D6 277.2/219.2; T 289.2/109.1, T-13C3 292.2/112.1; and AD 287.1/97.1, AD-13C3 290.2/100.1. The stock solutions of the isotope-labeled internal standards were made in methanol with a concentration of DHEA-D6 of 5 mg/mL, T-13C3 100 μg/mL, and AD-13C3 100 μg/mL, and these were further diluted to working solutions with a final concentration of DHEA-D6 of 45 ng/mL, T-13C3 of 15 ng/mL, and AD-13C3 of 15 ng/mL. The calibrating solution was prepared in a blank serum extracted on an Oasis HLB column (1 cc/30 mg, Waters). Specifically, the Oasis column was first equilibrated in methanol and water sequentially, and then loaded with 1 mL of double charcoal-stripped serum. The extract from the column was collected as the blank serum, and the standard materials

Table 1: Distributions and clinical characteristics of PCOS and control groups.

Groups	Number	Age	BMI (kg/m²)	F-G score	PRL (mIU/L)	E² (pmol/L)	SHBG (nmol/L)	LH/FSH
Type A	55	24.6±5.1	22.3±4.7	5.6±4.2*	267.4±102.5	138.7±59.2	57.4±33.1	1.9±1.5*
Type B	94	25.4±5.2	23.7±5.3	3.6±2.4	215.6±88.2	153.3±66.8	70.7±42.6	1.8±0.9*
Control	82	26.4±5.3	22.4±2.6	2.4±1.1	276.2±83.7	150.9±43.5	75.0±57.1	0.7±0.3

were dissolved in this blank serum in a series of concentrations to make calibrating and QC solutions. The concentrations of the calibration solutions for different analytes were 0.625, 1.25, 2.5, 20, 45, and 90 ng/mL for DHEA, and 0.125, 0.25, 1.5, 7.5, 15 and 30 ng/mL for AD and T. The concentrations of the QC solutions were 5, 50, and 500 ng/mL for DHEA, and 1, 10, and 100 ng/mL for AD and T. The sample was prepared by mixing 100 μL of serum sample/calibrator/QC solution with 200 μL of internal standard working solution, spinning for 2 min, and then shaking thoroughly at 700 rpm for 15 min at room temperature on a shaker. After that, the sample solution was centrifuged at 13000 × g for 10 min and the supernatant was collected for LC-MS analysis.

Data analysis

Data were analyzed automatically with manual confirmation. Statistical analysis was performed using SPSS 19.0. The means and standard deviations were calculated and compared between pairs of groups with ANOVA single factor analysis of variance or a Kruskal-Wallis test, with a test level $\alpha = 0.05$.

Results

Sample background, the F-G score and serum indexes

The patients' age, weight, BMI, and other physical parameters were not significantly different between the groups. The F-G score and serum data are summarized in (Table 1). F-G score in group A was significantly higher than that of either group B or controls ($P < 0.05$). The ratio of LH to FSH in groups A and B were both higher than the control group ($P < 0.001$). The levels of SHBG in group A were lower than in group B but without statistical significance ($P > 0.05$). The PRL and E2 showed no significant difference ($P > 0.05$).

The analysis of androgens in serum

We used a Chemiluminescent Immunoassay (CI) to analyzed the androgens T (T-CI) and DHEAS and obtained FTI based on T-CI and SHBG. We used LC-MS to determine concentrations of T (T-LC/MS), AD and DHEA. The linearity ranges of T-LC/MS, AD, and DHEA were 0.125–200 ng/mL, 0.25–200 ng/mL, and 1.25–1000 ng/mL respectively. The lower limit of quantification for T-LC/MS was determined as 0.125 ng/mL, AD as 0.125 ng/mL, and DHEA as 0.625 ng/mL. The recovery yield of all 3 hormones was within 85%–115% using the LC-MS method. Both intra-assay (same day) and inter-assay (different days) coefficients of variation were less than 15%. The concentrations of T-CI, DHEAS, T-LC/MS, AD, DHEA, and FTI in all the groups are summarized in (Figure 1).

1) The T-CI level of the PCOS patients in group A was 0.78 ± 0.26 ng/mL, higher than that in group B and controls, and it was higher in group B than in controls, both with statistical significance ($P < 0.001$). 2) The T-LC/MS of group A (0.50 ± 0.29 ng/mL) was higher than both group B and control group, and it was higher in group B than control group, both with statistical significance ($P < 0.05$). The T-LC/MS showed a lower value than T-CI with statistical significance ($P < 0.001$). 3) The DHEAS level of groups A (7.07 ± 2.40 μmol/L) and B were both higher than the control group ($P < 0.05$), and A was higher than B but without statistical significance. 4) The AD concentration of group A (1.09 ± 0.49 ng/mL) and group B (1.03 ± 1.29 ng/mL) were both higher than that of the control group (0.71 ± 1.38 ng/mL), both with $P < 0.001$, but no statistical difference shown between A and B ($P > 0.05$). 5) The DHEA level in group A was higher than in both group B and controls with no statistical significance ($P > 0.05$). 6) A higher value of FTI was seen in group A (9.12 ± 7.49) than in either group B or controls, and FTI was higher in group B (5.09 ± 5.0) than the control group, both with $P < 0.05$.

Discussion

To the best of our knowledge, no standard biochemical indexes have been available for the hyperandrogenism diagnosis and sub typing of PCOS [19]. Although the T concentration has been widely used as an indicator for hyperandrogenism when diagnosing PCOS, some PCOS patients do not have an elevated T level but do have higher concentrations of other androgens such as AD or DHEAS [15,20].

Our study analyzed multiple factors including T-CI, T-LC/MS, and FTI values in PCOS patients and a corresponding control group, and showed various levels in those groups. Based on our results, the levels of T-CI, T-LC/MS, and FTI in group A were higher than those in either group B or the control group, indicating that those factors are specific to hyperandrogenism and useful for PCOS diagnosis or sub typing. The relatively higher levels of T-CI, T-LC/MS, and FTI in group B than the control group demonstrates that these factors are involved in the development of the subtype disease of group B. We also observed that the increase of androgens corresponded with the disease development even though the level of serum T and DHEAS still remained in the normal range. These findings of elevated androgens were in alignment with the necessary criteria for PCOS diagnosis released by Androgen Excess Society (AES) in 2006 [2].

Both groups A and B showed higher levels of DHEAS and AD than the control group, demonstrating that DHEAS and AD can be used for the diagnosis of PCOS of both subtypes. It also suggests that these androgens could be involved in the disease mechanisms of both subtypes, but not specifically enough to differentiate the two subtypes.

Although the DHEA levels in both PCOS groups were higher than in the controls, the distinction was not statistically significant, thus DHEA can only be used as a reference factor for disease diagnosis.

In summary, our study used two different methods for measurement of serum T, namely CI and LC-MS. The T level obtained

from the LC-MS (T-LC/MS) was lower than from CI (T-CI), which was in agreement with a previous report [19]. A possible reason for the distinctive results could be a result of a cross-reaction with DHEAS in the CI. The measurement using LC-MS is more accurate and specific due to the inherent principle of this method [15,21]. LC-MS is known as an advanced technique for serum hormone measurement without the need for chemical derivatization or sample pre-treatment [22]. However, LC-MS systems and chemical reagents are usually expensive and instrument operation requires advanced technical training, therefore its application is limited to larger or more advanced laboratories. Further standardization is also needed to establish generic reference values between laboratories.

Conclusion

Overall, from our study, the androgen levels of the PCOS patient groups and controls all showed a wide range with much overlap (Figure 1). The heterogeneous characteristics of PCOS can also be seen from the clinical symptoms and the laboratory indexes. Although DHEAS and AD can be used for the diagnosis of PCOS, T and FTI are more specific to differentiate the PCOS subtypes.

Acknowledgment

This work was supported by the Wuhan Science and Technology Bureau under Grant 2013062301010815 and Wuhan Health and Family Planning Commission under Grant WX14C04.

References

1. Franks S. Polycystic ovary syndrome: a changing perspective. Clin Endocrinol (Oxf). 1989; 31: 87-120.
2. Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Futterweit W, et al. Positions statement: Criteria for Defining Polycystic Ovary Syndrome as a Predominantly Hyperandrogenic Syndrome: An Androgen Excess Society Guideline. J Clin Endocrinol Metab. 2006; 91: 4237-4245.
3. Okoroh EM, Hooper WC, Atrash HK, Atrash HK, Yusuf HR, Boulet SL, et al. Prevalence of polycystic ovary syndrome among the privately insured, United States. 2003-2008. Am J Obstet Gynecol. 2012; 207: 377.e1-377.e8.
4. Zawadaki R, Dockerty M. Diagnostic criteria for polycystic ovarian syndrome: towards a rational approach. In: Dunaif A, Given JR, Haseltine F, Merriam GR, editors, Current issues in endocrinology and metabolism: polycystic ovary syndrome. Boston. Blackwell Scientific. 1992; 377-384.
5. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril. 2004; 81:19-25.
6. Yildiz BO, Azziz R. The adrenal and polycystic ovary syndrome. Rev Endocr Metab Disord. 2007; 8: 331-342.
7. Goodarzi MO, Carmina E, Azziz R. DHEA, DHEAS and PCOS. J Steroid Biochem Mol Biol. 2015; 145: 213-225.
8. Zhao X, Ni R, Li L, Mo Y, Huang J, Huang M, et al. Defining hirsutism in Chinese women: across-sectional study. Fertil Steril. 2011; 96: 792-796.
9. Fauser BC, Tarlatzis BC, Rebar RW, Legro RS, Balen AH, Lobo R, et al. Consensus on women's health aspects of polycystic ovary syndrome (PCOS): the Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. Fertil Steril. 2012; 97: 28-38.e25.
10. Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. Consensus on women's health aspects of polycystic ovary syndrome (PCOS). Hum Reprod. 2012; 27: 14-24.
11. Li ZP, Zhang HY, Li H. Study of Electrochemiluminescence Immunoassay (ECLIA) in the determination of the levels of six kinds of hormones of normal women of childbearing age in menstrual cycle. Guizhou Medical Journal. 2003; 27: 794-795.
12. Ya-ping YE, Qi-zhi OU, Ai-ping QIN, et al. Detection of Serum Dehydroepiandrosterone Sulfate (DHEA-S) Levels in Reproductive Women and Its Clinical Significance. Reproduction & Contraception. 2013; 33: 755-759.
13. Dury AY, Ke Y, Gonthier R, Isabelle M, Simard JN, Labrie F. Validated LC-MS/MS simultaneous assay of five sex steroid/neurosteroid-related sulfates in human serum. J Steroid Biochem Mol Biol. 2015; 149: 1-10.
14. Bui HN, Struys EA, Martens F, de Ronde W, Thienpont LM, Kenemans P, et al. Serum testosterone levels measured by isotope dilution-liquid chromatography-tandem mass spectrometry in postmenopausal women versus those in women who underwent bilateral oophorectomy. Ann Clin Biochem. 2010; 47: 248- 252.
15. Barth JH, Field HP, Yasmin E, Balen AH. Defining hyperandrogenism in polycystic ovary syndrome: measurement of testosterone and androstenedione by liquid chromatography–tandem mass spectrometry and analysis by receiver operator characteristic plots. Eur J Endocrinol. 2010; 162: 611-615.
16. Fanelli F, Belluomo, Di Lallo VD, Cuomo G, De Iasio R, Baccini M, et al. Serum steroid profiling by isotopic dilution-liquid chromatography–mass spectrometry: Comparison with current immunoassays and reference intervals in healthy adults. Steroids. 2011; 76: 244-253.
17. Huang B, Han Z, Xu X, Cai Z, Jiang W, Ren Y. Simultaneous determination of 7 female sex hormones in essential oil by high performance liquid chromatography-tandem mass spectrometry with isotope dilution. Se Pu. 2011; 29: 20-25.
18. Søeborg T, Frederiksen H, Fruekilde P, Johannsen TH, Juul A, Andersson AM, et al. Serum concentrations of DHEA, DHEAS, 17α-hydroxyprogesterone, Δ4-androstenedione and testosterone in children determined by Turbo Flow-LC–MS/MS. Clin Chim Acta. 2013; 18: 95-101.
19. Keevil BG. How Do We Measure Hyperandrogenemia in Patients With PCOS? J Clin Endocrinol Metab. 2014; 99: 777-779.
20. O'Reilly MW, Taylor AE, Crabtree NJ, Hughes BA, Capper F, Crowley RK, et al. Hyperandrogenemia predicts metabolic phenotype in polycystic ovary syndrome: the utility of serum androstenedione. J Clin Endocrinol Metab. 2014; 99: 1027-1036.
21. 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.
22. Stanczyk FZ. Androgen Measurements: Methods, Interpretation, and Limitations. In: Azziz R, Nestler JE, Dewailly D, editors, Contemporary Endocrinology: Androgen Excess Disorders in women: Polycystic Ovary Syndrome and Other Disorders, Second Edition. Humana Press. 2006; 63-74.

Xiaoyan et al. © All rights are reserved

Citation: Xiaoyan D, Yanqin D, Yajun H, Qing X, Li L and Chunlin W. Androgen Profiling in Various Types of PCOS by Chemiluminescent Immunoassay in Combination with LC-MS. Austin J In Vitro Fertili. 2017; 4(2): 1036.