ROLE OF OVARY AND ADRENAL GLANDS IN HYPERANDROGENEMIA IN PATIENTS WITH POLYCYSTIC OVARY SYNDROME

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SUMMARY

Ovary is the main source of the hyperandrogenism in polycystic ovary syndrome (PCOS). Adrenal glands may also be involved in the palhogencsis of the development of PCOS. To investigate this possibility and to find out if buserelin test is able to distinguish PCOS patients from the patients with idiopatic hirsutism (IH), 29 women wilh PCOS, 21 women with IH, and 20 control subjects (CS) were subjected to ACTH and buserelin tests.

Basal and stimulated dehydroepiandrosterone sulfate (DHEA-S) and stimulated cortisol (F) levels after ACTH administration were significantly higher in PCOS group than in IH and CS groups ($p<0.0001$ and $p<0.05$, respectively). PCOS patients also possessed significantly higher basal and stimulated 17-hydroxyprogeslerone (17-OH P) leveis, including the peak IeVels ($p<0.02$), during buserelin testing when compared with IH patients and CS. There was no significant correlation between the ACTH stimulated and the buserelin stimulated peak 17-OH P values ($r=0.157$, $p>0.05$).

In conclusion, significantly higher basal and ACTH stimulated leveis of F and DHEA-S in PCOS compared with controls and patients with IH, reflect that adrenal hyperactivity plays a role in hyperandrogencmia seen in PCOS. The lack of the correlation betwcen ACTH and buserelin stimulated 17-OH P leveis makes it difficull to say that adrenal hypcractivity seen in PCOS is the result of the dysregulation of cylochrome P450c17-α enzyme. Buserelin test could distinguish at least some of the patients with PCOS from the other patients presenting with the common symptoms of hyperandrogenemia.

Key words: Polycystic ovary syndrome, ovary, adrenal gland, buserelin stimulation test

INTRODUCTION

Polycystic ovary syndrome (PCOS) remains a diagnostic challenge because there is no single defining test [1]. The clinical presentation must dictate the extent of the work-up. The typical PCOS patient has a history of irregular menses and appears hirsute [1]. Androgen excess is central to the pathophysiological changes and clinical expression of PCOS [2]. Although the ovary is the main source of the hyperandrogenism, a subset of women with PCOS also have adrenal androgen excess, usually to a mild degree [1]. So adrenal glands may also be involved in the pathogenesis of the development of PCOS [3,4]. Exaggerated secretion of ovarian androgens caused by abnormal regulation of cytochrome P450c17-α is thought to be the main reason of PCOS. Cytochrome P450c17-α is the enzyme that is important for both ovarian and adrenal steroidogenesis. This enzyme is encoded by a single gene on chromosome 10 and is expressed in both the adrenal glands and ovarian theca cells [5-7]. Some investigators have found evidence of dysregulation of the cytochrome P450c17-α enzymes that catalyze 17-α hydroxylase and 17-20 lyase activity in the adrenal and ovary [8,9], whereas other researchers dispute this finding [10,11]. An abnormal ovarian steroidogenic response to gonadotropin-releasing

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hormone (GnRH) analog is typical in patients with PCOS [8,12]. It is suggested that stimulation test with GnRH agonists may be useful to distinguish PCOS from other causes of hyperandrogenism [13], such as idiopathic hirsutism (IH). Supranormal elevation of 17-hydroxyprogcsterone (17-OH P) levels after GnRH agonists challenge has been reported both in postpubertal girls [14] and in adult patients with PCOS [8,13,15]. On the other hand, adrenal stimulation with adrenocorticotropin (ACTH) has been the principal challenge test for estimating the relative activity of adrenocortical enzymcs [16]. If the regulation of adrenocortical P450c17-α is abnormal, a positive corrclation would be expected between the adrenal 17-OH P response to ACTH and the ovarian 17-OH P response to GnRH agonist [17]. Both tests could be used in patients with PCOS to investigate this possibility.

In the current study, we aimed to investigate if adrenal glands play a role in hyperandrogenemia seen in PCOS. Besides we aimed to find out if buserelln (an GnRH agonist) test is able to distinguish PCOS patients from the patients with IH, and if abnormal regulation of cytochrome P450c17-α in both adrenal and ovary is responsible for the hyperandrogenemia seen in PCOS.

MATERIALS and METHODS

The study was approved by the Ethical Committee of Ankara University, School of Medicine, and informed consent was obtained from each woman.

Twenty-nine women with PCOS and twenty-one women with IH were randomly selected for study from our clinical population. The diagnosis of PCOS was made by the presence of three or more of the following criterias: oligo/amenorrhea, hyperandrogenemia, hirsutism, the presence of polycystic ovaries on pelvic ultrasound examination, and a serum luteinizing hormone (LH): follicle stimulating hormone (FSH) ratio >2. Oligomenorrhea was defined as menstrual cycles >35 days in length and amenorrhea was defined as absent of menstrual period in more than six months. All women with PCOS had hyperandrogenemia. Of those 29 patients with PCOS, 52% had oligo/amenorrhea. Without the presence of menstrual disturbances and any other signs or symptoms of hyperandrogenemia except hirsutism in the patient population, the diagnosis of IH was made. All patients had hirsutism (modified Ferriman-Gallwey score >8) [18].

The control group consisted of 20 normal women similar in age and body mass index to the subjects with PCOS and IH. Ali had regular menses every 26-32 days, and no evidence of hirsutism.

Late-onset congenital adrenal hyperplasia, Cushing's syndrome, androgen-secreting tumors, and hyperprolactinemia were excluded by appropriate tests. None of the subjects in any group had received any hormonal medication at least eight weeks before the study. All the subjects were studied in the follicular phase (days 3-8) and in the event that the patient was amenorrhcic, random a day. All patients and control subjects underwent ACTH and buserelin stimulation tests following basal determlnations of LH, FSH, estradiol (E_2), progesterone (P), ACTH, cortisol (F), prolactin (PRL), total and free testosterone (tT and fT), dehydroepiandrosterone sulfate (DHEA-S), 17-OH P, and 11-desoxycortisol (11-DOC).

ACTH stimulation test was performed in ali subjects by administration of a single i.m. 1 mg synthetic ACTH-(1-24) (Synacthen amp, Ciba, Basel, Switzerland) in the fasting state in the supine position. Venous blood was withdrawn through an indwelling catheter at 0, 6 and 8 hours for determination of serum 17-OH P, 11-DOC, F and DHEA-S. After

testing, the serum was separated and stored at -20°C until assayed.

Buserelin test was performed after an overnight fast. An intravenous catheter was inserted in an antecubital vein and baseline blood sample was drawn. Immediately after the basal blood sample collection, 1 mg buserelin (Suprefact, Hoechst, Germany) was given subcutaneously and blood samples were drawn for 17 OH P, LH, and FSH analysis, at 6th, 12th, and 24th hours following buserelin administration. The serum samples were stored at at -20°C until they were assayed.

Both ACTH stimulated and buserelin stimulated 17-OH P leveis were determined so as to evaluate the relationship between adrenal and ovarian source of hyperandrogenemia.

Serum DHEA-S and F were measured by electrochemiluminescence immunoassay (Elecsys Systems 1010/2010/ Modular Analytics E170, Roche Diagnostics GmbH, Mannheim, Germany; normal ranges were 70-300 µg/dl in females and 5-25 µg/dl, respectively). 17-OH P, 11-DOC, and fT were measured by radioimmunoassay, using ^{125}I RIA kits (17-α-OH P kit: ICN Biomedicals, Inc. Diagnostics Division 3300 Hyland Avenue Costa Mesa, CA 92626; normal range 0.4-1.02 ng/ml in the follicular phase- fΓ kit: DSL-4900, Diagnostic systems laboratories, Texas, USA; normal range 0.29-3.18 pg/ml). Serum LH, FSH, ACTH, and PRL were measured by immunometric assay (IMMULITE 2000, Diagnostic Products Corporation Los Angeles, CA, USA; normal ranges were 2.4-12.6 mIU/ml and 3.5-12.5 mIU/ml in follicular phase, 10-55 pg/ml, and 4.1-18.4 ng/ml, respectively). E2, P and tT were measured by competitive immunoassays by using available kits (IMMULITE 2000, Diagnostic Products Corporation Los Angeles, CA, USA; normal ranges 26-161 pg/ml, 0.12-1.7 ng/ml in follicular phase, and 6-82 ng/dl, respectively).

The intra-assay coefficients of variation (CVs) for the measurement of 17-OH P were 12.3%, 7.8%, and 8.3% for low, medium, and high leveis, respectively. The inter-assay CVs for 17-OH P were 12.9%, 9.8%, and 12.8%. For 11-DOC intra-assay CVs were 2.1%, 5.9%, and 4.3% and inter-assay CVs were 13.7%, and 11.6%. intra-assay CVs of F were between 3.2% and 2.2%, and inter-assay CV was 20%. For the measurement of DHEA-S, intra-assay CVs for low, medium, and high leveis were as follows: 2.8%, 2.4%, and 1.7%, respectively.

For statistical analysis Student's paired t-test and One way ANOVA were used for the comparisons within the same group and between the groups respectively. Pearson correlation analysis was also carried out. A P value of <0.05 was regarded as statistically significant.

RESULTS

Table 1 shows the clinical and basal hormonal characteristics of the women with PCOS, women with IH, and normal controls.

LH/FSH ratio was higher in PCOS patients than patients with IH and controls. Both tT and fT concentrations, DHEA-S and ACTH leveis were also found to be higher in patients with PCOS than in patients with IH and control subjects. Besides, basal F leveis were higher in PCOS and in IH when compared with controls. In patients with PCOS, basal 17-OH P and 11-DOC leveis were significantly higher comparing with control subjects as well. But basal LH, FSH, E_2, P, and PRL leveis did not differ between groups.

Table 2 shows the basal and stimulated values of F, 17-OH P, 11-DOC, and DHEA-S during ACTH stimulation test

Following ACTH administration, inerease in F, 17-OH P, 11-DOC, and DHEA-S leveis,

Table 1. Clinical and basal hormonal characteristics of the women with polycystic ovary syndrome (PCOS), women with idiopatic hirsutism (IH), and control subjects (CS) (mean±SD)

Variable		PCOS (n: 29)	IH (n: 21)	CS (n: 20)	p*
Age	(year)	22.37±3.79 (16-30)	24.00±4.90 (17-30)	22.15±3.28 (17-30)	NS
BMI	(kg/m^2)	26.04±7.70 (17.0-40.0)	24.96±4.87 (16.5-35.0)	24.13±6.67 (17.2-38.7)	NS
Hirsutism score		10.68±3.38 (9-25)	10.71±5.83 (8-28)	3.75±1.86 (1-6)	<0.01[a]
LH	(IU/ml)	6.55±6.00	6.10±1.96	4.96±2.43	NS
FSH	(IU/ml)	5.75±1.59	5.41±2.45	7.39±6.33	NS
LH/FSH		1.78±1.57	0.78±0.44	0.93±0.59	<0.01[b]
E_2	(pg/ml)	76.50±54.85	67.36±35.60	58.84±31.89	NS
P	(ng/ml)	0.94±0.42	2.89±4.95	1.33±2.12	NS
tT	(ng/ml)	68.31±26.65	41.49±16.17	36.70±15.91	<0.001[b]
fT	(pg/ml)	4.84±1.88	1.86±0.76	1.54±0.80	<0.001[b]
PRL	(ng/ml)	24.31±13.54	21.16±11.10	19.30±8.69	NS
F	(µg/ml)	23.38±7.52	20.62±5.22	14.44±4.03	<0.001[a]
ACTH	(pg/ml)	43.43±27.39	28.88±12.43	20.89±9.68	<0.001[b]
DHEA-S	(µg/ml)	329.80±120.16	207.05±88.18	203.65±63.56	<0.001[b]
17-OH P	(ng/ml)	2.55±1.54	1.98±1.68	1.33±0.83	<0.02[c]
11-DOC	(ng/ml)	5.46±3.65	3.97±2.06	2.71±1.57	<0.004[c]

** One way ANOVA, NS: Not significant*
a. PCOS and IH groups are significantly different than CS
b. PCOS group is significantly different than IH and CS
c. Significant difference between PCOS and CS

including the peak levels, were all significantly higher at any time point comparing with the mean basal values in all groups ($p<0.001$ for all the comparisons). In spite of the fact that, basal and stimulated 17-OH P and 11-DOC leveis did not differ between groups, basal and stimulated DHEA-S levels, including the peak level, and stimulated F leveis at 6th and 8th hours after ACTH administration, were all found to be significamly higher in PCOS group than in IH and CS groups. Besides, basal and stimulated peak F levels were significantly higher in PCOS group when compared with the controls.

Table 3 shows the basal and stimulated values of LH, FSH, and 17-OH P during buserelin stimulation test.

Following buserelin administration, increase in LH and FSH levels, including the peak levels, were all significantly higher at any time point comparing with the mean basal values in all groups ($p<0.001$ for all the

Table 2. Comparison of the basal and ACTH stimulated values of F, 17-OH P, 11-DOC, and DHEA-S (intergroup and intragroup differences are available) (mean±SD).

		PCOS (n: 29)	IH (n: 21)	CS (n: 20)	p*
F_0	(µg/dl)	23.06±5.75	21.46±5.54	17.61±5.76	<0.009$
$F_{6\ hour}$	(µg/dl)	66.17±22.25 a	52.02±12.97 a	52.49±10.30 a	<0.008µ
$F_{8\ hour}$	(µg/dl)	66.16±22.40 a	54.71±15.26 a	55.48±12.66 a	<0.05µ
F_{peak}	(µg/dl)	69.73±22.74 a	57.11±15.36 a	56.10±11.18 a	<0.02$
17-OH P_0	(ng/ml)	2.81±2.02	2.06±1.26	2.44±2.08	NS
17-OH $P_{6\ hour}$	(ng/ml)	6.63±5.62 a	4.45±3.21 a	6.96±4.90 a	NS
17-OH $P_{8\ hour}$	(ng/ml)	8.61±7.55 a	5.82±3.89 a	9.17±6.80 a	NS
17-OH P_{peak}	(ng/ml)	9.29±7.27 a	6.15±3.84 a	9.45±6.61 a	NS
11-DOC_0	(ng/ml)	3.70±2.31	3.09±1.48	3.43±2.78	NS
11-$DOC_{6\ hour}$	(ng/ml)	10.24±4.22 a	8.65±5.06 a	9.14±5.14 a	NS
11-$DOC_{8\ hour}$	(ng/ml)	11.31±5.18 a	10.77±7.29 a	9.71±2.52 a	NS
11-DOC_{peak}	(ng/ml)	13.10±4.48 a	11.62±6.62 a	11.09±3.97 a	NS
DHEA-S0	(µg/dl)	313.14±98.07	224.86±117.52	218.44±101.54	<0.004µ
DHEA-$S_{6\ hour}$	(µg/dl)	444.35±132.56 a	299.89±107.31 a	305.44±118.58 a	<0.0001µ
DHEA-$S_{8\ hour}$	(µg/dl)	473.46±138.63 a	335.42±134.86 a	314.11±109.44 a	<0.0001µ
DHEA-S_{peak}	(µg/dl)	486.92±141.60 a	343.31±137.83 a	329.38±116.47 a	<0.0001µ

**One way ANOVA, NS: Not significant.*
$. Significant difference between PCOS and CS groups
µ. PCOS group is significantly different than the IH and CS groups
a P<0.001, compared with the basal value in the same group with Paired samples t test

comparisons). 17-OH P rcached the highest level 24 hours after buserelin administration in all the groups. Buserelin stimulated peak 17-OH P levels were already significantly higher in three groups comparing with their mean basal values. Basal and stimulated LH, FSH levels did not differ between groups, except stimulated FSH levels 24 hours after buserelin. At that time point, FSH leveis were found to be significantly higher ln controls than in PCOS and IH patients. However, PCOS patients possessed significantly higher basal and stimulated 17-OH P levels, including the peak levels, during buserelin testing when compared with IH patients and control subjects. Two standard deviation above the mean peak value in our 20 normal women has been accepted as the cut-off level of peak value [2.54+(2x1.66)=5.86 ng/ml]. Only five out of 29 (17.24%) PCOS women had an increased response of 17-OH P. Their peak serum 17-OH P levels were >5.86 ng/ml. None of 21 women with IH had peak serum 17-OH P level above this value. But according to these results, it can be concluded that buserelin test may not distinguish all PCOS patients from the other causes of hirsutism or hyperandrogenic states.

Figure 1 shows the comparison of the median peak and the median net incremental val-

Table 3. Comparison of the basal and buserelin stimulated values of LH, FSH, and 17-OH P (intergroup and intragroup differences are available) (mean±SD).

		PCOS (n: 29)	IH (n: 21)	CS (n: 20)	p*
LH_0	(IU/ml)	6.32±5.49	6.13±5.96	5.31±3.98	NS
$LH_{6\ hour}$	(IU/ml)	68.67±47.34 [a]	61.30±36.43 [a]	63.73±38.68 [a]	NS
$LH_{12\ hour}$	(IU/ml)	40.57±23.34 [a]	41.27±24.71 [a]	43.37±20.17 [a]	NS
$LH_{24\ hour}$	(IU/ml)	30.17±21.90 [a]	34.74±18.08 [a]	43.90±32.01 [a]	NS
LH_{peak}	(IU/ml)	70.02±46.27 [a]	61.30±36.43 [a]	69.33±41.66 [a]	NS
FSH_0	(IU/ml)	5.61±1.44	5.93±2.02	8.64±11.41	NS
$FSH_{6\ hour}$	(IU/ml)	31.88±10.98 [a]	28.86±11.20 [a]	37.48±34.72 [a]	NS
$FSH_{12\ hour}$	(IU/ml)	21.51±8.49 [a]	21.49±8.03 [a]	30.84±28.84 [a]	NS
$FSH_{24\ hour}$	(IU/ml)	13.15±4.39 [a]	14.89±4.31 [a]	23.98±17.89 [a]	<0.002 §
FSH_{peak}	(IU/ml)	31.88±10.98 [a]	29.04±10.98 [a]	38.48±34.37 [a]	NS
17-OH P_0	(ng/ml)	2.77±1.85	1.52±0.94	1.44±0.87	<0.001 μ
17-OH $P_{6\ hour}$	(ng/ml)	2.54±1.77	1.54±0.79	1.48±0.84	<0.007 μ
17-OH $P_{12\ hour}$	(ng/ml)	2.68±1.84	1.58±1.17	1.47±0.93	<0.006 μ
17-OH $P_{24\ hour}$	(ng/ml)	3.62±2.54 [c]	2.31±1.61 [c]	2.40±1.63 [c]	<0.05 μ
17-OH P_{peak}	(ng/ml)	4.07±2.56 [a]	2.56±1.46 [a]	2.54±1.66 [b]	<0.02 μ

**One way ANOVA, NS: Not significant.*
§. CS group is significantly different than PCOS and IH groups
μ. PCOS group is significantly different than the IH and CS groups
a P<0.001, compared with the basal value in the same group with Paired samples t test
b P<0.01, compared with the basal value in the same group with Paired samples t test
c P<0.05, compared with the basal value in the same group with Paired samples t test

ues of ACTH stimulated 17-OH P in three groups.

Neither the peak leveis, nor the net ineremental values in ACTH stimulated 17-OH P differed between groups.

Figure 2 shows the comparison of the median peak leveis and the median net ineremental values of buserelin stimulated 17-OH P in three groups.

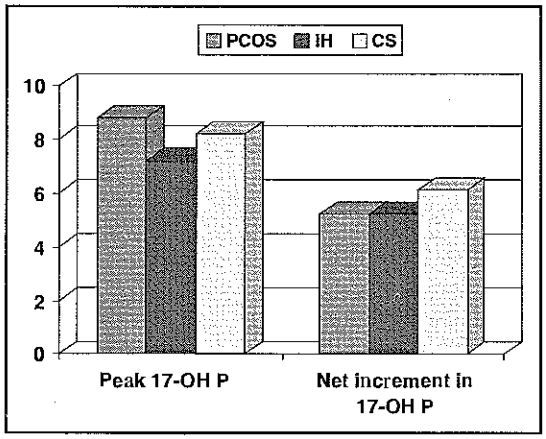
Although buserelin stimulated 17-OH P peak leveis were found to be significantly higher in PCOS patients when compared with the patients with IH and control subjects, no such significant difference was found for the net ineremental values of buserelin stimulated 17-OH P between groups.

Correlation analysis revealed that there was no significant correlation between the ACTH stimulated and the buserelin stimulated peak 17-OH P values (r=0.157, p>0.05), and between the net inerements in 17-OH P leveis (r=-0.083, p>0.05) after ACTH stimulation and buserelin stimulation tests in patients with PCOS.

DISCUSSION

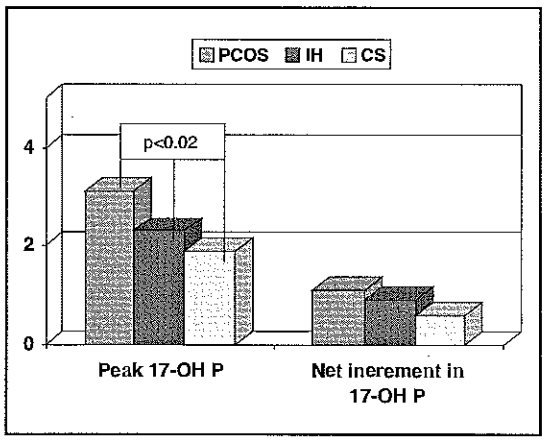
Polycystic ovary syndrome is probably conditioned by heterogeneous elements. Al-

Figure 1. Comparison of the median peak and the median net incremental values of ACTH stimulated 17-hydroxyprogesterone (17-OH P) in three groups.



PCOS: Polycystic ovary syndrome
IH: Idiopatic hirsutism
CS: Control subjects

Figure 2. Comparison of the median peak and the median net incremental values of buserelin stimulated 17-hydroxyprogesterone (17-OH P) in three groups.



PCOS: Polycystic ovary syndrome
IH: Idiopatic hirsutism
CS: Control subjects

though it is characterized by menstrual abnormalities, hyperandrogenism, hirsutism and obesity it still remains a common disorder and its pathogenesis is stili unclear. The main source of hyperandrogenism seen in PCOS is the ovary [19]. The exaggerated 17-OH P response after acute GnRH agonist nafarelin [9] or buserelin [13] stimulation also suggested that the elevated circulating 17-OH P concentrations are a product of ovarian secretion. On the other hand, numerous investigators have found elevated androgen production by the adrenal glands in patients with PCOS and have suggested that the adrenal glands may play a role in the genesis of PCOS [3,4,20]. Similar with the previous findings, in our study, we have also found some evidence showing adrenal hyperactivity in patients with PCOS, such as higher basal F, 11-DOC [21,22], DHEA-S [23], and 17-OH P [23] levels when compared with normal controls. Though ACTH stimulated 17-OH P and 11-DOC levels did not differ between PCOS patients and patients with IH or control subjects, significantly higher stimulated levels of F and DHEA-S in patients with PCOS during ACTH testing were in favour of the possibility that adrenal glands play a role in excess androgen production in a subgroup of patients. Contradictory to the previous study done in the same area [17], we could not find any significant difference for the ACTH stimulated 17-OH P levels between groups. Moreover, ACTH stimulated and buserelin stimulated 17-OH P peak levels or net incremental values were not correlated. These results were against the hypothesis that abnormal regulation of P450c17-α funetion in both the adrenal cortex and ovary could explain the hyperandrogenic funetion of both glands.

Azziz et al. studied the 17 hydroxylase and 17,20-lyase produets and precursors before and after ACTH stimulation in hyperandrogenic women and their data did not support the concept that adrenocortical excess in hyperandrogenism is the result of cytochrome P450c17-α dysregulation [11]. In another study, Azziz et al. suggested that hyperactivity may result from the overproduction of other non-ACTH adrenal-stimulated factors [21]. Others have suggested that the adrenal androgen excess and the exaggerated secretion of steroidogenic precursors result from a generalized hyperresponsivcness of the adrenal cortex to ACTH and other factors as yet to be determined [24,25]. Keleştimur et al.

also suggested that the leading cause of adrenal hyperandrogenism in PCOS is adrenal androgen hyperresponsiveness to ACTH [26]. Higher basal ACTH leveis seen in patients with PCOS may be the result of this association in our study.

Our findings indicate that PCOS patients have an increased 17-OH P response to the GnRH agonist buserelin. Similar to the previous study done in the same area [27], we have found that the concentrations of 17-OH P after buserelin stimulation in the patients with PCOS were significantly higher than the control women or than the patients with IH. Şahin et al. propossed that 17-OH P response to buserelin is a useful test in the diagnosis of PCOS [13]. In contrast, sensitivity of buserelin test was found to be low in our study. So buserelin test can not be used to diagnose ali PCOS patients. No significant positive correlation between the peak plasma 17-OH P after buserelin administration and basal serum LH level was found in hyperandrogenic women in our study, suitable with the results of another previous study [8]. A recent study demonstrated that PCOS women with 17-OH P hyperresponsiveness to GnRH agonist testing have a remarkably consistent pattern of steroid responses regardless of whether they have elevated basal LH leveis [28]. It has been suggested that the stimulation of 17-α hydroxylase/17,20-lyase by LH may be augmented by specific intraovarian and hormonal factors such as inhibin, insulin-like growth factor-1 and insulin and ovarian hyperandrogenism can result from abnormal modulation of ovarian androgen responsiveness to LH [8]. In conclusion, significantly higher basal and ACTH stimulated leveis of F and DHEA-S in PCOS compared with controls and patients with IH, reflect that adrenal hyperactivity also plays a role in hyperandrogenemia seen in PCOS. Because of the lack of the correlation between ACTH stimulated and buserelin stimulated 17-OH P leveis, it is hard to say that adrenal hyperactivity seen in PCOS is the result of the dysregulation of cytochrome P450c17-α enzyme. Instead some other factors, such as hyperresponsiveness to ACTH could be the underlining cause in adrenal adrogen overproduction. On the other hand, ovary is the main source of androgen excess in patients with PCOS and buserelin test which is an GnRH analogue could distinguish at least some of the patients with PCOS from the other patients presenting with the common symptoms of hyperandrogenemia.

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