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Relationship between Hormonal Changes and Folliculogenesis in Polycystic Ovarion Syndrome Women

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Abstract
Polycystic ovarian syndrome (PCOS) is a syndrome of oligomenorrhea, hirsutism, and polycystic ovary with chronic anovulation and varying degrees of androgen excess. It manifests itself in a variety of clinical ways and 55%–75% of patients with PCOS are infertile due to chronic anovulation. Despite substantial effort, the etiology and pathogenesis of PCOS and polycystic ovaries (PCO) in women remain unknown. Whereas, the present study planned to find out the relationship between the hormonal changes and foliculogenesis of the normal and PCOS patients. The patients were selected from infertility clinics and classified as control (group I), untreated PCOS (group II) and treated groups (III and IV). Treated groups were received 1500 mg metformin per day (500 mg three times a day) for 6–8 weeks, at the end of this period, the patients in the group III were given 100 mg clomiphene citrate and group IV patients received 2.5 mg letrozole from 3-7 days of their menstrual cycle. The serum Insulin, LH, FSH and Testosterone levels were significantly ($p< 0.001$) decreased in after the treatment (group III and group IV). Follicles were classified as primordial, transitional primary, classic primary, secondary, and Graafian. The total number of different types of follicles were significantly ($p < 0.001$) increased in PCOS than normal ovaries. The present study outcome suggests that the primary follicle growth is abnormally slow in PCOS and the dynamics are reflected in classic primary follicles. From this evidently established the correlation between the hormonal changes and folliculogenesis.

Key words: Folliculogenesis. Polycystic ovarian syndrome. Letrozole

INTRODUCTION:

Polycystic ovary syndrome (PCOS) is a common endocrinopathy affecting an estimated 6–10% of women worldwide [1]. It represents the most common endocrinopathy of women of reproductive age. Although descriptions of polycystic ovaries date back to the early 1700s, variable degrees of virilization, menstrual abnormalities, and bilaterally enlarged polycystic ovaries form the basis for the definition of PCOS [2]. In addition to the clinical manifestations of infertility and virilization, metabolic consequences of PCOS include obesity, type II diabetes, hyperlipidemia, hypertension, and cardiovascular disease [3].

To date, there is no overall consensus on the definition of PCOS or the criteria used for diagnosis. Current understanding for diagnosis encompasses the 1990 National Institutes of Health criteria [4], Rotterdam 2003 criteria [5], and most recently the Androgen Excess-PCOS (AE-PCOS) Society criteria [1]. According to the AE-PCOS Society's working definition, PCOS is a disorder of androgen excess or hyperandrogenism with the following clinical findings: hyperandrogenism (hirsuitism and/or hyperandrogenemia), ovarian dysfunction (oligo-anovulation and/or polycystic ovaries), and the exclusion of other androgen excess–related disorders [1].

The area of active investigation in PCOS has been the ovary [6-7]. This analysis has focused primarily on the granulosa and theca interstitial cells of the small Graafian follicles that accumulate in the cortex of PCOS ovaries. At the differentiation level, the PCOS Graafian follicles appear to be up-regulated as demonstrated by the presence of supraphysiological levels of bioactive FSH in the microenvironment [8], overexpression of FSH receptors in the granulosa cells [9], hypersensitivity to FSH stimulation [10], and enhanced capacity of the theca to produce androgens in response to LH and insulin stimulation [11]. These properties
can be contrasted with a reduced capacity for follicle cell proliferation, which in turn causes the PCOS Graafian follicle to stop growing. The arrest of Graafian follicle development is paradoxical because one would expect that the high levels of FSH bioactivity in follicular fluid and the super FSH responsiveness of the granulosa cells would lead to the selection of multiple dominant follicles [12].

Now in the research filed the overlooked question in PCOS is when during folliculogenesis follicle growth and development become abnormal. Based on studies in laboratory animals, it is clear that

growing follicles can be disrupted in their growth and development long before they reach the more advanced Graafian stage [13]. For example, growth differentiation factor (GDF-9) [14] and bone morphogenetic protein (BMP-15) [15] produced by oocytes have profound growth and differentiation effects on the small preantral follicles. Evidence also points to a key role of Oocyte growth factors in regulating cytodifferentiation, including FSH receptor expression and action [16], cumulus expansion and theca androgen production [17].

In the present study is planned to compare the ovarian structure and assay the insulin, LH, FSH changes in control, baseline and treated patients. Retrospectively compared and Find out the correlation between the ovarian morphology and hormonal changes.

MATERIALS AND METHODS:

STUDY POPULATION:

All patients between the age of 27 and 37 years who attended a infertility clinic with a suspicion of PCOS (specifically, complaining of infertility, menstrual dysfunction or dermatological problems), were included in the study. The study sample was collected from various infertility clinics in Tamilnadu, India. The study was approved by the Scientific Ethics Committee, Coimbatore, Tamilnadu. An informed written consent was obtained from each patient before entering the study. All patients with oligomenorrhoea (a cycle length of 45 days or six periods per year) or amenorrhoea, who also had evidence of hyperandrogenism (a hirsutism score 7, according to Ferriman and Gallway) and an elevated serum testosterone level, were diagnosed as having PCOS, after all the other causes of hyperandrogenism had been excluded. Subjects treated with hormonal medications within 3 months were also excluded

Healthy normal cycled women conclude as a control (group I), untreated PCOS patients selected for (group II), metformin – clomiphene citrate treated patients as (group III) metformin–letrozole treated patients as (group IV).All patients of III and IV groups received 1500 mg metformin (Glucophage, Merck, West Drayton, UK) per day (500 mg three times a day) for 6–8 weeks. After the end of this period, the patients in the group III were given 100 mg clomiphene citrate and group IV patients received 2.5 mg letrozole [Femara, Novartis, Quebec, Canada) from 3-7 days of their menstrual cycle

SAMPLE COLLECTION:

All blood samples were obtained in the morning between 0800 and 0900 h after an overnight fast and resting in bed. In particular, at baseline blood samples were obtained during the early proliferative phase (second through third day) of the P-induced withdrawal uterine bleeding (for the cases) or the spontaneous uterine bleeding (for the controls), whereas throughout the study, they were obtained randomly in anovulatory PCOS patients and during the early proliferative phase of the spontaneous uterine bleeding in both controls and ovulatory PCOS patients. Blood samples (5 ml) were collected into tubes containing EDTA after a 12-h fast and a 30-min resting period in the supine position and immediately centrifuged at 4 C for 20 min at 1600 x g, and plasma samples were stored at –20 C.

HORMONAL ASSAY:

The Plasma insulin levels were measured by chemiluminescent enzyme immunoassay (Immulite 2000; Diagnostic Products Corporation). Total testosterone (T) was measured by a chemiluminescent immunometric method (Immulite 2000; Diagnostic Products Corporation). LH, FSH were measured by chemiluminescent enzyme immunoassay (Immulite 2000; Diagnostic Products Corporation, Los Angeles, CA, USA).

TISSUE SAMPLE COLLECTION:

A total of 20 ovaries were used in these investigations. Normal ovaries were obtained from 12 regularly cycling women (aged 25–39 yr) at various stages of the menstrual cycle. The surgeries were for nonovarian gynecological reasons. None was receiving exogenous hormones. Ovaries were obtained from five patients (aged 27–37 yr) with PCOS as defined by chronic anovulation and hyperandrogenism. The clinical data of these PCOS subjects have been reported previously [18, 19]. Briefly, the subjects had oligomenorrhea or amenorrhea and were hirsute. All had undergone laparotomy with the finding of PCO confirmed by histological examination. Serum hormone determinations revealed significant elevations of mean LH, total T, androstenedione, and dehydroepiandrosterone sulfate. Ovaries were obtained from three patients

with polycystic-appearing ovaries as diagnosed by the pathologist at the time of surgery. The PCO diagnosis was confirmed by a histological examination of the ovaries carried out in our laboratory. None of the clinical histories of the PCO patients was available. The protocol was

approved by the Scientific Ethics Committee, Tamilnadu, and written informed consent had been obtained from each individual.

FOLLICLE COUNTING:

The ovary samples were fixed in 10% neutral buffered formalin, paraffin embedded, sectioned, and stained with hematoxylin and eosin. Follicles were scored in a random 10 µm section from each ovary. Precisely where in the ovary the sections were obtained was not determined.Two different observers scored follicles independently with similar results. Follicles were classified into four groups as described [20]: primordial (the oocyte was surrounded by a single layer of squamous granulose cells); primary (the oocyte was surrounded by a single layer of mixed squamous and cuboidal or a single layer of cuboidal granulosa cells); secondary (the oocyte was surrounded by two to eight layers of granulose cells but no antrum was present); and small Graafian. The majority of Graafian follicles did not contain an oocyte in the sections examined. Therefore, all Graafian follicles (healthy and atretic) were scored using the antrum as a marker (Such follicles measured between 0.5 and approximately 8mmin diameter). The total number of points, *e.g.* the area in megapixels/mm2, was then used in the sample analysis.

STATISTICAL ANALYSIS:

The data are reported as the mean +/- SD or the median, depending on their distribution. The differences in quantitative variables between groups were assessed by means of the unpaired t test. One way Analysis of variance (ANOVA) was performed followed by multiple comparisons using the scheffe test. Comparison of a variable between two groups was assessed by Mann-Whitney Test. A p value of <0.05 using a two-tailed test was taken as being of significance for all statistical tests. All data were analyzed with a statistical software package (SPSS, version 13.0 for windows).

RESULTS:

The population consisted of 200 subjects (Female population) divided into four groups was selected. Patients visited with infertility problem in various hospitals in various cities, Tamil Nadu, India with suspected PCOS patients was selected as source of population based on the inclusion and exclusion criteria. The control subjects were selected based on inclusion and exclusion criteria. They were not receiving any drugs at the time of the study. General health characteristics such as age, body weight, BMI, hirsutism, menstrual status were investigated by a self-administered questionnaire.

The demographic characteristics like body weight, BMI, hirsutism, menstrual cycle status were significantly increased in after treatment (data's not shown). Our previous publication shows[22], After the treatment, Biochemical levels also reverted to the normal range.

The serum Insulin, LH, FSH, and Testosterone levels were assayed in both the treatment groups (III & IV) compared with group I and group II (Table I). After the treatment with Metformin - Clomiphene citrate and Metformin- Letrozole Insulin, LH, FSH and testosterone levels were significantly (P< 0.001) suppress and reverted near to the normal range.

The mean number of follicles per ovary section was used to evaluate differences in follicle populations between PCOS and normal. These data are showed in Fig. 1. The total number of follicles and Primordial, Primary, Secondary, Graafian follicles were significantly increased (P < 0.001) in PCOS when compared with controls patients.

Table 1. Comparision of hormonal changes in control, baseline and treated groups.

Parameters	GroupI Control	Group II Untreated PCOS	Group III Met - CC	Group IV Met - let
Insulin µu / ml	7.4 ± 1.6	16.4 ± 5$$$	9.0 ± 1.7###***	8.2 ±1.3**‡‡‡, †
LH U/ L	5.4 ±2.1	25.6± 6.8$$$	15.2 ± 3.8###***	11.2 ± 2.0***‡‡‡, †††
FSH U/ L	7.2 ± 1.4	12.2± 2.0$$$	10.9 ± 1.9###**	9.9 ±1.7***‡‡‡, †
Testosterone nmol / L	0.9 ± 0.48	4.3 ± 2.0$$$	3.1 ± 1.4###**	1.2 ± 0.56**‡‡‡, †

Values are given as mean ± SD from fifty subjects in each group
Group IV compare with Group I significant at the present -*P <0.5,**P<0.01,***P<0.001, NS –Non significant
Group III compare with Group I significant at the present -#P <0.5,##P<0.01,###P<0.001, NS –Non significant
Group II compare with Group I significant at the present -$P <0.5,$$P<0.01,$$$P<0.001, NS –Non significant
Group III compare with Group II significant at the present -*P <0.5, **P<0.01, ***P<0.001, NS –Non significant
Group IV compare with Group II significant at the present -‡P <0.5,‡‡P<0.01, ‡‡‡P<0.001, NS –Non significant
Group IV compare with Group III significant at the present -†P <0.5, ††P<0.01, †††P<0.001, NS –Non significant

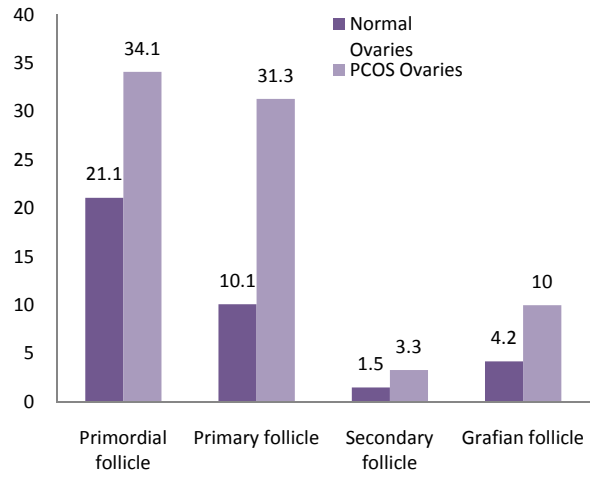

Fig1. The mean level of Primordial, Primary, Secondary and Graafian follicles in section of ovaries from normal and PCOS patients.

DISCUSSION:

A key question in reproductive medicine concerns the nature of the abnormalities or defects that lead to PCOS women. The diversity in the endocrine profiles of women with PCOS has led to the search for some unifying principle to explain the etiology and pathogenesis of this syndrome. Here, this study provides new insight into the dynamics of folliculogenesis in PCOS. Our findings support to the relationship with hormonal changes and morphology of folliculogenesis.

Hyperinsulinemia plays a pivot role in development of hyperandrogenemia in PCOS patients. Insulin directly stimulates androgen production from theca-cells [21, 22]. Sex hormone binding globulin level in the liver is decreased, and the level of free testosterone is increased [23]. At the same time, hyperinsulinemia increases IGF-1 by inhibiting insulin-like growth factor (IGG-1) binding protein, produced by the liver, and thus, androgen production from theca-cells is stimulated [24]. Metformin helps to decreases fasting glucose level by decreasing hepatic glucose output. Its use in PCOS patients, corrects the response to oral glucose tolerance, thus decreasing insulin level. [25, 26].In our study suggest that after the treatment insulin levels are deceased to the normal range.

In this study, observed improvements with regard to the clinical effects of androgens including hirsutism and acne. These findings agree with most, but not all, previous reports [27]. These effects may be related to a decreased LH and improvement of hyperinsulinemia, resulting in lower ovarian androgen production [28]. Aromatase enzyme has direct effect on the ovaries and increase follicular sensitivity to FSH. The level of ovarian aromatase is low in these patients. Multiple small ovarian follicles are due to high androgen level. In addition, androgens increase FSH receptors, and therefore increase FSH sensitivity. Aromatase inhibitors cause growth of one or more ovarian follicles by increasing FSH or deceasing estrogen production [41].

In this study, the metformin – letrozole group patients LH, FSH and Testosterone levels were significant reduction after the treatment period**.** In a previous study of 10 subjects, Fruzzetti et al. [27] observed a statistically non-significant trend toward lower LH level. Since hyperinsulinemia and hyperandrogenism may alter the secretion of gonadotrophins in favor of an increase in LH, these drugs were lower LH secretion by reducing insulin and/or androgen levels [29].

PCOS ovaries would be expected to contain fewer primordial follicles. Nor were atretic, preantral follicles identified in the control and PCOS ovaries. Taken together, these results support the hypothesis that primary follicles in PCOS ovaries are growing more slowly than normal. Supporting this view is the recent observation by Webber *et al.* [30] that the proportion of early growing (primary) follicles is higher in anovulatory and ovulatory women with PCOS ovaries compared with that in normal ovaries. Further support for this concept comes from a classical study performed by Hughesdon [31] on full-thickness Stein-Leventhal ovarian wedges wherein the numbers of primary, secondary and Graafian follicles were found to be twice normal. Thus, these two reports are consistent with our conclusion that the rate of primary follicle growth is reduced in PCOS.

The second possibility is the effect of excessive ovarian androgen production. Experiments in monkeys demonstrate that exogenous androgens increase the number of classic primary follicles [32]. The mechanism is unclear but appears to be mediated by androgen receptors expressed in the granulosa cells [33]. In both normal [34] and PCOS [35] ovaries, androgen receptors are expressed in granulosa cells of preantral follicles. Thus, a model for describing changes in the rate of folliculogenesis in PCOS, which takes into account the local effect of increased ovarian androgens on the accumulation of classic primary follicles, could be proposed.

The third possibility is that increased LH secretion influences the growth of primary follicles. It is well recognized that the rate of LH release is increased in women with PCOS [36] and that increased plasma LH contributes to increased androgen production by the theca interstitial cells [37]. That this LH alteration might be a part of the mechanism of primary follicle build-up in PCOS ovaries comes from a rather startling study in mice [38]. LH-stimulated theca androgen production is implicated in increasing the number of classic primary follicles in monkey ovaries. PCOS subjects in our study were exposed to increased insulin action, which could also act to promote theca androgen production. If true, then elevated insulin levels could be related to the mechanism of the primary follicle accumulation in PCOS.

Another possibility is FSH bioactivity. Indeed, the ability of FSH to directly stimulate preantral follicle growth supports this possibility [39]. Interestingly, PCOS granulosa cells are supersensitive to FSH stimulation [8, 10], and up-regulation of FSH receptors appears to contribute to this phenomenon [9]. In this scenario, androgens have been shown to significantly increase FSH receptor mRNA abundance in primate granulosa cells [40], although its relevance to PCOS awaits confirmation in functional studies.

In summary, the present study points to defects in the ability of primary follicles to grow normally in PCOS. From this evidently established the correlation between the hormonal changes and folliculogenesis. But it emphasizes the importance of investigating further the possible role of recruitment in this pathology.

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