Relationship between the follicular distribution pattern of polycystic ovaries and the degree of menstrual disturbance and serum sex steroid levels

Polikistik overlerin foliküler dağılım paterni ile menstrüel bozukluk derecesi ve serum seks steroid düzeyleri arasındaki ilişki

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Abstract

Objective: This study aimed to examine the associations between follicular distribution pattern (FDP) in polycystic ovaries and menstrual disturbances in women with infertility.

Materials and Methods: A retrospective review of patients was performed (n=73). Ultrasound images from cycle day 2-5 of a spontaneous or progestin induced menstrual cycle were reviewed. Ovaries were classified as polycystic ovarian morphology (PCOM) if they contained ≥12-follicles measuring 2-9 mm in diameter. Images of PCOM ovaries were classified as having a peripheral cystic pattern (PCP) with follicles arranged at the periphery of the ovary, or general cystic pattern (GCP) if follicles were dispersed heterogeneously throughout the ovarian stroma. Menstrual disturbance was assessed by questionnaire, and oligomenorrhea was defined as cycles >35 days in length.

Results: PCP was more strongly associated with menstrual irregularity that GCP. 94% of subjects with bilateral PCP-experienced oligomenorrhea compared with 65% of women with a unilateral PCP ovary [odds ratio (OR) 9; p<0.05]. 29% of women with bilateral GCP ovaries experienced menstrual disturbances, less than bilateral PCP (OR 36; p=0.002), but similar to unilateral PCP (OR 3; p=0.07). Serum testosterone and luteinizing hormone (LH) levels were significantly correlated with the ovarian FDP.

Conclusion: There is a relationship between menstrual irregularity or certain types of serum steroids and ovarian morphology. It remains unknown if morphology, testosterone or LH causes the menstrual disturbance or if they are co-initiated by an intervening factor.

Keywords: Polycystic ovary syndrome, oligomenorrhea, ovarian follicle

Öz

Amaç: Bu çalışmanın amacı, infertiliteli kadınlarda polikistik overlerde foliküler dağılım paterni (FDP) ile menstrüel bozukluklar arasındaki ilişkileri incelemektir.

Gereç ve Yöntemler: Hastaların geriye dönük incelemesi yapıldı (n=73). Spontan veya progestin ile indüklenen adet döngüsünün 2-5 günlerindeki ultrason görüntüleri incelendi. Yumurtalıklar 2-9 mm çapında ≥12 folikül içeriyorsa polikistik over morfolojisi (PCOM) olarak sınıflandırıldı. PCOM yumurtalıklarının görüntüleri, foliküler yumurtalığın çevresinde düzenlenmiş ise periferik kistik patern (PCP) veya foliküler yumurtalık stroması boyunca heterojen olarak dağılmışsa genel kistik patern (GCP) olarak sınıflandırıldı. Menstrüel bozukluk anket ile değerlendirildi ve oligomenore >35 gün uzunluğundaki sikluslar olarak tanımlandı.

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Bulgular: PCP adet düzensizliği ile GCP'den daha güçlü bir şekilde ilişkiliydi. Tek taraflı PCP yumurtalığı olan kadınların %65'ine kıyasla iki taraflı PCP'li deneklerin %94'ü oligomenore yaşamaktaydı [risk oranı (RO) 9; p<0,05]. İki taraflı GCP yumurtalıkları olan kadınların %29'u menstrüel bozukluklar yaşamaktaydı. Bu oran tek taraflı PCP yumurtalıkları olan kadınlardaki orana benzerken (RO 3; p=0,07), iki taraflı PCP yumurtalıkları olan kadınlardaki orandan düşüktü (RO 36; p=0,002). Serum testosteron ve luteinleştirici hormon (LH) seviyeleri, yumurtalık FDP ile önemli ölçüde ilişkiliydi.

Sonuç: Menstrüel düzensizlik ile belirli serum steroidleri ve over morfolojisi arasında bir ilişki vardır. Morfoloji, testosteron veya LH'nin adet düzensizliğine neden olup olmadığı veya araya giren bir faktör tarafından sürecin başlatılıp başlatılmadığı bilinmemektedir.

Anahtar Kelimeler: Polikistik over sendromu, oligomenore, yumurtalık folikülü

Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder of unknown, multiple etiologies with a clinical syndrome characterized by hyperandrogenism, oligoamenorrhea, and infertility[1]. The clinical findings of PCOS are highly variable, making the diagnostic criteria of the condition controversial[2-4]. In 1990, the National Institutes of Health (NIH) consensus statement on diagnostic criteria for PCOS excluded ovarian morphology[5]. However, the 2003 international consensus on PCOS diagnosis held in Rotterdam proposed the inclusion of ovarian morphology into the diagnostic criteria of PCOS[6]. Although the Rotterdam criteria for polycystic ovarian morphology (PCOM) include the presence of ≥12 follicles measuring 2-9 mm in diameter and/or increased ovarian volume (>10 cm³) in a single or both ovaries[2], there remains considerable debate over how to best define the ovarian appearance in PCOS[1]. Lujan et al.[7] reported that the follicle number per ovary (FNPO) threshold of 26 follicles resulted in the best sensitivity and specificity to distinguish women with PCOS from healthy controls. Therefore, in 2014, the Androgen Excess Society and Polycystic Ovary Syndrome Society guidelines recommended using FNPO of ≥25 for the definition of PCOM when using newer ultrasound technology with maximal ovarian follicle resolution[8]. Most recently, the 2018 international evidence-based guideline for the assessment and management of PCOS recommended using a FNPO of at least 20 follicles[9].

While the number of follicles required for the definition of PCOM has been debated and updated, there has been less discussion about the patterns of follicle distribution (FDP) within a PCOM ovary. The Adam's criteria of PCOM on ultrasound initially described 10 or more follicles arranged in a peripheral pattern around a dense core of stroma, called the "string of pearls" pattern[10]. This pattern became known as one of two classes of PCOM ovaries based on the distribution of follicles in the ovary: A peripheral cystic pattern (PCP). The second class, called a general cystic pattern (GCP), describes ovaries with multiple small follicles occupying the entire parenchyma of the ovary[11]. Takahashi et al.[12] examined differences between women with PCP and GCP ovaries and reported that serum androstenedione and the luteinizing hormone (LH)/Follicle stimulating hormone (FSH) ratio was significantly higher in women with PCP rather than GCP ovaries. This finding suggests an endocrinological difference between PCP and GCP ovaries. Furthermore, different underlying pathophysiological processes of disturbed folliculogenesis may result in different patterns of FDP in PCOM ovaries[13,14]. Earlier studies investigating the ultrasound characteristics of PCOM revealed that a peripheral distribution of ≥10 follicles around the midpoint of the ovary was a highly sensitive criterion for the diagnosis of PCOS[15].

Therefore, this study aimed to evaluate whether, in women with infertility and PCOM, the PCP FDP when measured at the ovarian midpoint is more strongly associated with menstrual irregularity compared with women GCP FDP and the relationship between FDP and serum hormone levels.

Materials and Methods

A retrospective chart review was conducted of 123 cycles of in vitro maturation (IVM) during a two-year period at the McGill University Reproductive Centre. After excluding additional cycles in patients with multiple cycles and incomplete records, 73 subjects remained for analysis. Subjects were required to have ≥12 follicles measuring 2-9 mm in diameter in at least one ovary (per the Rotterdam Criteria)[6]. Subjects with a dominant follicle ≥10 mm or an ovarian cyst on either ovary were excluded from the study. Furthermore, subjects were required to have no: Clinical or biochemical evidence of thyroid abnormalities (0.39< serum thyroid-stimulating hormone <3.0 μIU/mL); hyperprolactinemia (am fasting serum prolactin <26 ng/mL); hypothalamic pituitary dysfunction or ovarian failure (1.4< FSH >20 IU/L and estradiol >20 pg/mL); ovarian and adrenal androgen-secreting tumors (total testosterone <200 ng/mL and DHEAS <800 μg/dL); and non-classical congenital adrenal hyperplasia (am fasting 17-hydroxy-progesterone <2 ng/mL). Finally, subjects were excluded from analysis if they used hormones, clomiphene citrate, aromatase inhibitors, or other medications (including insulin sensitizing medications), which could have affected the follicle count or distribution in the previous 90 days.

All patients who underwent IVM had a baseline pelvic ultrasound by a certified technician (Quebec diplomat in Radiology techniques), and serum blood tests (total testosterone, free testosterone, LH, and FSH) on day 2-5 of a natural or progesterone provoked cycle (medroxyprogesterone, 10 mg daily taken orally for 5-14 days). The number of follicles was documented and a copy of the ultrasound images was included in the chart. Follicle count and distribution was assessed by subjective evaluation of ultrasound images by two physician investigators and images were categorized into three groups based on ovarian morphology:

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1) Normal morphology: (<12 follicles) without a predominantly peripheral distribution,

2) PCP: ≥12 follicles peripherally distributed around a dense stromal core for at least 50% of the ovarian diameter.

3) GCP: ≥12 follicles located throughout the ovary and not more than 49% in a peripheral distribution.

Each physician was blinded to the other's diagnoses. If the diagnoses of the two physicians differed, a third physician was consulted and then agreement of the diagnosis of two of three physicians was then accepted. All physicians were gynecologists with extensive experience in trans-vaginal ultrasonography. The third physician was consulted only for two cases.

Menstrual regularity was determined by questioning the patient on the duration of most menstrual cycles at the time of initial presentation to the fertility clinic. Subjects with cycles less frequent than 35 days, for 75% of cycles, were considered oligomenorrheic.

Statistical Analysis

Statistical analysis was performed using Stats Direct. Chi-square tests with Yates correction and odds ratios with Fisher's exact tests were also used. ANOVA was used to compare group continuous data, while chi-squared tests were used to compare the categorical data. Tukey's Post-hoc testing was used for post ANOVA comparisons. For the case of the chi-square test, if a number was zero in one category a one was substituted. Spearman's correlation coefficient was used to compare relationships in different groupings with the continuous demographic data. Data were compared with odds ratios and confidence intervals. Data are presented as N and percentage or mean ± standard deviation.

Committee for the Protection of Human Subjects approval of the study was obtained. None of the authors have any conflicts of interest.

Results

The demographic, data, rates of menstrual disturbance and serum steroid levels, stratified for follicular distribution pattern are reported in Table 1. When considering the demographics, the groups were similar for age and BMI. The oldest woman in this study was 36 years of age. However, the group with one GCP was more likely to have conceived previously, suggesting a relationship between follicular distribution pattern and fertility potential. All subjects had follicle counts of at least twelve in one ovary.

When considering serum hormone levels (Table 1) patterns were discernable based on the follicular distribution pattern and whether that distribution pattern occurred in one or both ovaries. The serum total and free testosterone levels decreased in a linear fashion from women with two PCP, to one PCP ovary, to two GCP ovaries, then to one GCP ovaries. A similar decrease in serum LH and the LH to FSH ratio was noted in the relationship with follicular distribution patterns. These findings demonstrated statistically significant correlations between ovarian morphology and serum total testosterone (r=-0.63, p<0.01), serum free testosterone (r=-0.58, p=0.01), serum LH levels (r=-0.66, p<0.01), and the LH/FSH ratio (r=-0.45, p<0.05). (For this analysis groupings were performed in the following order two PCP, one PCP, two GCP, one GCP).

Fifty-three percent of women were oligomenorrheic (39/73), defined as having menstrual cycles longer than thirty-five days, at least 75% of the time. Sixteen subjects had PCP morphology

Table 1. Demographics, menstrual data and serum steroid levels of the patients stratified by the ovarian morphology

Ovarian morphology	2 PCP ovaries n=16 (Group 1)	1 PCP ovary n=21 (Group 2)	2 GCP ovaries n=17 (Group 3)	1 GCP ovary n=36 (Group 4)	p-value
Age (Years)	30±1.8	30±1.7	30±1.9	31±1.8	0.09
BMI (kg/m^2)	23.9±3.2	23.2±2.9	22.8±2.7	23.5±3.1	0.74
Nulliparous	16 (100%)	20 (95%)	17 (100%)	13 (36%)	0.0001
Total serum testosterone (nmol/L)	2.2±0.3	1.9±0.3	1.6±0.2	1.4±0.3	0.0001*
Serum free testosterone (nmol/L)	1.84±0.5	1.6±0.6	1.1±0.5	0.9±0.3	0.0001**
Serum LH (IU/L)*	7.9±0.9	6.7±1.0	5.7±1.3	5.5±1.2	0.0001*
Serum FSH (IU/L)*	6.5±1.2	6.5±0.9	6.7±1.4	7.2±1.4	0.13
LH/FSH ratio	1.2±0.2	1.0±0.3	0.85±0.3	0.76±0.2	0.0001***
#with Oligomenorrhea	15 (94%)	13 (62%)	5 (29%)	6 (17%)	0.0001

Note: serum bloods tests were performed on cycle day 2 to 5 of a spontaneous or Medroxyprogesterone acetate induced menstrual cycle and on the same day as the ultrasounds. PCP: Peripheral cystic pattern, GCP: General cystic pattern, BMI: Body mass index, LH: Luteinizing hormone, FSH: Follicle stimulating hormone, Data were compared using ANOVA. p<0.05 statistically significant.

Post-hoc testing (only statistically significant differences are indicated, p<0.05):

*Group 1> Group 2, Group 1> Group 3, Group 1> Group 4, Group 2>Group 3, Group 2>Group 4

**Group1>Group3, Group1>Group4, Group2>Group3, Group2>Group4

***Group1>Group3, Group1>Group4, Group2>Group4

in both ovaries, 94% (15/16) of which were oligomenorrheic. Twenty-one subjects had one ovary with PCP morphology with the second ovary having <12 follicles and 62% (13/21) of these patients were oligomenorrheic. Seventeen subjects had GCP morphology in both ovaries, 29% (5/17) of which were oligomenorrheic. Thirty-six subjects had one ovary with GCP morphology with the second ovary having <12 follicles and 17% (6/36) of them were oligomenorrheic. Interestingly, none of the subjects had a PCP on one ovary and a GCP on the other ovary.

The odds ratio of menstrual disturbance comparing either of the FDPs to the other three types is presented in Table 2. PCP ovaries were more strongly associated with menstrual disturbances than were GCP ovaries. Compared to having bilateral GCP ovaries (29% oligomenorrheic), having bilateral PCP ovaries conferred 36 times increased odds of experiencing menstrual disturbance compared to women with a unilateral PCP ovary (62% oligomenorrheic), women with bilateral PCP ovaries (94% oligomenorrheic) were 9 times more likely to experience menstrual irregularities. Women with a unilateral PCP were more likely to experience menstrual irregularity (62%) than women with bilateral GCP (29%), although this was not statistically significant (p=0.07). There was no statistical difference in the rates of menstrual disturbances in women with bilateral GCP ovaries (29% oligomenorrheic) compared to women with a unilateral GCP ovary (17% oligomenorrheic). Women with a unilateral PCP ovary (62% oligomenorrheic) were 8 times more likely to experience menstrual disturbances than women with a unilateral GCP ovary (17% oligomenorrheic).

Discussion

The main objective of the current study was to evaluate whether the FDP in PCOM ovaries of women with infertility can be useful in predicting the severity of menstrual irregularities, specifically oligo and anovulation. From a clinical standpoint, it is important to understand how different PCOM morphologies are related to the severity of the disease itself. Using a subset of women undergoing IVM at the McGill University Reproductive Centre who were known to have PCOM and infertility, we evaluated whether the FDP of their ovaries correlated with the degree of menstrual irregularity experienced by the patient. We noted a significantly higher correlation between PCP ovaries and oligomenorrhea than with GCP ovaries and oligomenorrhea. Furthermore, none of the subjects in the study were found to have one ovary with each type of distribution pattern; they either had only PCP or GCP ovaries, not both.

Women with a unilateral PCOM ovary showing a PCP FDP were more likely to experience menstrual irregularity than women with bilateral GCP FDP. This finding suggests that the FDP seen in the ovary is a more significant prognostic factor for the severity of clinical presentation than is the bilaterality of PCOM ovaries. Furthermore, PCP FDP was more likely to be associated with increased total and free testosterone levels, as well as increased LH/FSH ratio. These findings are in agreement with previous studies illustrating a relationship between FDP and hyperandrogenism, supporting the assertion that PCP and GCP ovarian morphologies may differ in their endocrine and pathophysiological processes[12-14].

Christ et al.[16] previously studied the FDP and compared it with reproductive and metabolic features of PCOS to assess the use of sonographic features to predict the severity of PCOS. In contrast to the results presented in this study, Christ et al.[16] concluded that FDP was not associated with any reproductive marker or metabolic parameter associated with PCOS. A potential explanation for this discrepancy may be due to the cohort of subjects used for each study. Our study population

Table 2. Odds ratio and 95% confidence interval of having menstrual irregularity based on ovarian morphology when compared to the index group on the left

Ovarian morphology	2 PCP n=16	1 PCP n=21	2 GCP n=17	1 GCP n=36
2 PCP n=16	----	9 (CI 0.9 to 84) p<0.05	36 (CI 4 to 351) p=0.0002	75 (CI 8 to 681) p=0.0001
1 PCP n=21	9 (CI 0.9 to 84) p<0.05	----	3 (CI 0.9 to 13) p=0.07	8 (CI 2 to 28) p<0.01
2 GCP n=17	36 (CI 4 to 351) p=0.0002	3 (CI 0.9 to 13) p=0.07	----	2 (CI 0.5 to 8) p=0.29
1 GCP n=36	75 (CI 8 to 681) p=0.0001	8 (CI 2 to 28) p<0.01	2 (CI 0.5 to 8) p=0.29	----

Please note the ---- occurred in boxes because subjects could not be compared to themselves. PCP: Peripheral cystic pattern, GCP: General cystic pattern, CI: Confidence interval, p<0.05 statistically significant

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was that of infertile patients undergoing IVM cycles for known PCOM. Unlike in Christ et al.'s study,[16] which used the NIH definition of PCOS, evidence of hyperandrogenism was not used as an inclusion criterion in our study[4,16].

When compared to women with a unilateral PCP ovary, women with bilateral PCP were nine times more likely to experience menstrual irregularities. While there was no difference seen in the rates of menstrual irregularity in women with bilateral GCP ovaries compared to women with a unilateral GCP ovary, a trend was present, which may become statistically significant in a larger study. The presence of a PCP FDP is suspected to occur secondary to a stromal core with increased density and vascular blood flow, which could push the ovarian follicle peripherally. Stromal density and vascular flow have previously been shown to predict the severity of PCOS as they are correlated with levels of ovarian hyperandrogenism[1,17-20]. Unlike PCP ovaries, GCP ovaries do not display increased stromal density and may suggest less androgenic disturbance in the patient, even when both ovaries are found to be multicystic. This would provide a plausible explanation for the difference in menstrual disturbances seen in women with two PCP ovaries, verses one PCP ovary, verses any GCP ovaries.

Study Limitations

Our study has limitations. First, this study is based on the retrospective and subjective assessment of FDP by static, baseline ultrasound images. Although the investigators reviewing the images did not have information regarding the cycle lengths of the subjects and any disagreement between investigators was resolved with a third assessor, we cannot completely rule out the low possibility of a classification bias. Second, our analysis was restricted to a relatively homogenous population of women undergoing infertility assessment and treatment. We cannot determine whether the difference in ovarian morphology represents different, similar syndromes grouped into PCOS or a continuum determined by increased severity of the disease in one population. Anti-Müllerian hormone levels would have been interesting to have; however, they were unavailable as they were not being routinely performed in our clinic at the time the patients were evaluated.

Conclusion

This study affirms the importance of assessing FDP in a population of women with known PCOM and undergoing infertility treatment. A PCP FDP may be useful in identifying a subset of women who are more likely to have worse menstrual disturbances. The mechanism of the relationship between menstrual irregularity and ovarian morphology requires further study to better understand the pathophysiology of this disease.

Ethics

Ethics Committee Approval: Committee for the Protection of Human Subjects approval of the study was obtained.
Informed Consent: Retrospective study.

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