Endometrial Thickness as a Predictor of Endometrial Hyperplasia in Infertile Patients with Polycystic Ovary Syndrome

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Abstract

Background: Women with polycystic ovary syndrome (PCOS) are at higher risk of developing endometrial hyperplasia (EH). This study determined the prevalence of EH among women with infertility due to PCOS and assessed the predictive value of endometrial thickness (ET) measurement by trans-vaginal scan (TVS).

Study Type & Population: This was a prospective study on infertile women with and without PCOS in which clinical data, hormonal profile, ET and endometrial biopsy (EB) for histopathological examination were collected.

Methods: Thirty-seven women with PCOS and 23 women without PCOS presenting with infertility and/or abnormal uterine bleeding underwent TVS, hysteroscopy, laparoscopy and EB.

Results: The overall prevalence of EH was 23.3% while in PCOS group: 18.3%. The mean ET (14.8 mm) was significantly higher among patients with EH (t = −2.74, P value = 0.009). The lower value of ET among women with EH was 10 mm. A cut-off point of 9.5 mm was set. ET of >9.5 mm had 92.9% sensitivity and 51.85% specificity for the presence of EH. Women with ET ≥ 9.5 mm were 1.28 times more at risk of EH than women with ET < 9.5 mm. ET was positively correlated with insulin resistance (r = 0.439, P = 0.007).

Conclusion: ET ≥ 9.5 mm predicts EH in infertile women with PCOS, with a high degree of sensitivity and a moderate degree of specificity. In PCOS patients with oligomenorrhea or irregular cycles, the risk of EH is higher than women with regular cycles.
Keywords
Polycystic Ovary Syndrome, Endometrial Hyperplasia, Endometrial Thickness, Oligomenorrhea, Trans-Vaginal Scan, Infertility

1. Introduction

Polycystic ovary syndrome (PCOS) is the most common multisystem endocrine disorder affecting 5% - 10% of women during their reproductive age [1]. PCOS is a heterogeneous condition with different clinical phenotypes [2], its pathophysiology appears to be due to polygenic and endocrinal changes which are characterized by hyperandrogenism and chronic anovulation [1] [3] [4] [5]. The endometrium in women with PCOS is affected by hormonal and metabolic abnormalities which contribute to some menstrual abnormalities observed in those women [6]. The endometrium of PCOS patients has high levels of insulin-like growth factor-1 (IGF-1) activity [7], decreased concentrations of sex hormone-binding globulin (SHBG) [8], up-regulation of endometrial aromatase, hyperandrogenemia and hyperinsulinemia. Consequently, those molecular changes increase the potential for neoplastic changes within the endometrium [9]. Anovulation is common among women with PCOS, hence, the progesterone levels are within suboptimal or absent effects over the endometrium, and the tissue is in a state of a relative over-response to the proliferative effects of estrogen (E2), where its levels were found to be comparable to the follicular phase levels [10]. These relatively high levels of E2 prohibit the endometrium from enduring the sequential changes in gene expression and its associated endocrine processes. Insulin resistance and hyperinsulinemia especially in obese patients may trigger the development of PCOS in genetically predisposed individuals. Therefore, in PCOS, the prolonged unopposed oestrogen, hyperinsulinemia, elevated free IGF-1 and androgens may further augment mitogenic activity within endometrial cells by activating mitogen-activated protein kinase (MAPK), leading to high prevalence of hyperplasia and possible transformation to endometrial cancer [9] [11].

Endometrial Hyperplasia (EH) is an abnormal proliferation of endometrial stroma and glands and represents a spectrum of endometrial changes ranging from glandular atypia to frank neoplasia, which only can be determined by a biopsy. Up to one-third of endometrial carcinomas are assumed to be preceded by hyperplasia [12]. The prevalence of endometrial hyperplasia in women with PCOS varies from 1% to 48.8% [13], and anovulation related to PCOS is a recognized common risk factor for endometrial hyperplasia, with or without cytologic atypia [6] [14] [15] [16] [17] [18]. On the other hand, studies suggested that the prevalence of endometrial cancer among PCOS cases is not higher than those of the general population [13] [19]. The prevalence of endometrial cancer in women with PCOS is estimated to be around 20% - 37% [20], which increases even more in obese women with PCOS [21]. Conversely, a recent review stated
that this risk is overestimated [22].

There is some evidence showing that there is an increase in endometrial thickness throughout the menstrual cycle in infertile patients with PCOS, when compared to infertile patients without PCOS [23]. Several studies have attempted to find a predictive cut-off value to measure endometrial thickness in cases of patients with EH [16] [24] [25]. Therefore, endometrial thickness should be assessed in women with long standing PCOS in order to detect those who are at risk of developing hyperplasia with or without atypia. Though, it is still unclear at this stage to determine which PCOS patients need endometrial biopsy for the purpose of intervention to avoid any long term complications.

The aim of this study was to investigate the prevalence of endometrial hyperplasia in infertile women with PCOS compared to those without PCOS, and to evaluate the predictive value of endometrial thickness, using U/S scan, to identify cases with EH in patients with PCOS, as previous studies are regionally related, few and controversial.

2. Methods

This prospective study recruited consecutive infertile women with and without PCOS who agreed to participate in this study. Patients were diagnosed as PCOS according to the selection criteria stated by the Revised 2003 Consensus [26]. The criteria to diagnose PCOS included: 1) oligo-anovulation, 2) clinical or biochemical hyperandrogenism (total testosterone > 2 SD), 3) ultrasound feature of polycystic ovaries with 12 or more follicles of 2 - 9 mm diameter in one ovary. Clinical variables such as age, body weight and height were assessed in all subjects during their visit in the outpatient department. Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m²). All PCOS patients were on insulin sensitising agent treatment for at least three months and all of them went through lifestyle modification and ovulation induction by clomiphene citrate (4 months) and monitored gonadotrophin therapy (2 months). PCOS patients included in this study were planned for hysteroscopy and laparoscopic ovarian drilling. The control group consisted of women with infertility undergoing diagnostic hysteroscopy and laparoscopy. Because infertility was the primary problem, none had received any contraception for more than one year.

All patients underwent hysteroscopy for uterine cavity evaluation and endometrial biopsy in the first 5 days of follicular phase, for this reason it took us long time to coordinate for the admission of patients to be in the right date of menstrual cycle. For microscopic evaluation, endometrial specimens obtained by curettage were fixed in 10% neutral phosphate buffered formalin solution. Following dehydration in ascending series of ethanol (70%, 80%, 96%, 100%), tissue samples were cleared in xylene and embedded in paraffin. Tissue sections of 5 µm were stained with hematoxylin-eosin (H-E). A minimum of ten fields within three different levels of sectioning for each specimen were evaluated blindly and separately and diagnosed according to the WHO classification for endometrial
hyperplasia [27].

2.1. Endometrial Thickness Measurement

After a routine pelvic examination, endometrial thickness was measured using a trans-vaginal transducer of frequency of 3 - 7.5 MHz (Hitachi, Aloka). Measurement was performed on the same day of admission to the hospital, which was in the first 5 days of follicular phase by scanning the uterus longitudinally to identify the plane in which the endometrium is best outlined. Endometrial thickness (double layer) was considered as the widest distance from the reflective interface between the endometrium and the myometrium of opposite sides on a sagittal view of the uterus reported in millimeters (mm).

2.2. Ovarian Assessment

The revised Rotterdam criteria were used for the diagnosis of PCOS [26] [28]. The PCO cases were identified using ultrasound either, as those with an ovary that contains twelve or more follicles with a diameter of 2 - 9 mm each, or as those with an increased ovarian volume (>10 cm³ without concomitant cysts) on at least one ovary, to be selected for the study. As none of the patients were using oral contraceptives, their ultrasound examination was performed during the first 5 days of the menstrual cycle (the early follicular phase). The scans were done trans-vaginally using a computed sonography system with a transducer of frequency of 3 - 7.5 MHz (Hitachi, Aloka). After identification of the ovaries, the size of the ovary was measured in three orthogonal planes. Ovarian volume was calculated using the formula for a prolate ellipsoid. The total number of follicles in each ovary was counted. Measurements of the largest and smallest follicles were taken in their maximum diameters and recorded in mm. Follicles were counted on the frozen images of two non-overlapping planes in the longitudinal section of each ovary. The presence of a single PCO was sufficient to provide diagnosis [28].

2.3. Hormonal Assessment

Blood samples were collected from all participants after been instructed not to eat, drink or smoke for the last 12 hours. All patients reported that they didn’t consume alcohol ever. A 10-cc sample of blood was drawn in plain-top tubes for subsequent hormonal analysis which was in the same day of the collection. Blood was analyzed for LH (units per L), FSH (units per L), fasting blood glucose (mg/dL), fasting insulin (microunits per mL), sex hormone binding globulin (SHBG) (nanomoles per L). Normal insulin sensitivity was defined by insulin levels < 12 mU/mL, indices of the homeostasis model assessment (HOMA-IR) [29] was calculated using the equation: HOMA-IR = Fasting insulin (μU/mL) × Fasting glucose (mg/dL)/405 [30] with HOMA-IR > 2.5 mU/mL/L as cut-off abnormal value [31] [32]. This model is used for the estimation of insulin sensitivity from the steady glucose and insulin concentrations measured under fasting
conditions [33]. Generally, it is well correlated with clamp techniques and has been used in many studies to assess IR in individuals with/without diabetes mellitus or PCOS [33]. Moreover, it is accepted as the standard method of IR detection in epidemiologic studies [34].

2.4. Tissue Storage

All tissue obtained were stored for future analysis and archived at the Department of Anatomy and Histology at Mutah University. Patient data were kept at the Department of Obstetrics & Gynecology at Mutah University with restricted access.

3. Statistical Analysis

The data were normally distributed based upon the value of Kolmogorov-Smirnov test of normality (P value-0.2), therefore, parametric statistical analysis was used. Clinical parameters between 2 groups were compared using a chi-square test (χ²) and the Student’s t-test. Receiver operating characteristics (ROC) curve analysis was used to find the predictable clinical factor for endometrial disease and decide the cut-off values. Data analysis was performed by using the software Statistical Package for Social Sciences (SPSS) Version 16 (SPSS Inc 2008). The level of significance was taken at P < 0.05. Both bivariate and multivariate techniques (simple and multiple logistic regression analysis) were used as a statistical tool in this study. Logistic regression (LR) analysis was performed in order to obtain adjusted estimates of odds ratios (AORs) of risk factors for endometrial hyperplasia. The health outcome-related variables were selected using a Likelihood Ratio (LR) backward approach.

4. Ethical Approvals and Patient Consent

Informed written consent was obtained from all patients. The study was approved by The Ethics and Scientific Research Committees in Mu‘tah University (2010-2).

5. Results

5.1. Patient’s General Characteristics

From October 2011-June 2016, a total of 67 cases of infertile women with and without PCOS were initially enrolled in this study. Four cases had inadequate endometrial specimens for histopathological evaluation, and were therefore excluded from the study; three cases were also excluded due to loss of relevant records, so the final number was 60 patients. All participants were Jordanian females with mean age of 25.3 ± 4.8 years. The patients were divided into two groups: women with PCOS (Rotterdam Criteria) n=37 (61.7%), and women without PCOS n=23 (38.3%) cases. The main features of both groups are shown in (Table 1). Both groups were comparable regarding age and BMI. The mean
thickness of the endometrium was 12.2 mm ± 5.05 and 10.87 mm ± 4.82 in Table 1. Main features comparing PCOS and non-PCOS groups.

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>N</th>
<th>Mean (Std. deviation)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, years</strong></td>
<td>With PCOS</td>
<td>37</td>
<td>25.6 (4.9)</td>
<td>0.368</td>
</tr>
<tr>
<td></td>
<td>Without PCOS</td>
<td>23</td>
<td>23.9 (3.8)</td>
<td></td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td>With PCOS</td>
<td>37</td>
<td>26.4 (2.0)</td>
<td>0.947</td>
</tr>
<tr>
<td></td>
<td>Without PCOS</td>
<td>23</td>
<td>26.5 (1.2)</td>
<td></td>
</tr>
<tr>
<td><strong>Endometrial thickness, mm</strong></td>
<td>With PCOS</td>
<td>37</td>
<td>12.2 (5.05)</td>
<td>0.505</td>
</tr>
<tr>
<td></td>
<td>Without PCOS</td>
<td>23</td>
<td>10.9 (4.8)</td>
<td></td>
</tr>
<tr>
<td><strong>Insulin resistance (HOMA-IR)</strong></td>
<td>With PCOS</td>
<td>37</td>
<td>3.0 (1.0)</td>
<td>0.024*</td>
</tr>
<tr>
<td></td>
<td>Without PCOS</td>
<td>23</td>
<td>2.1 (1.0)</td>
<td></td>
</tr>
</tbody>
</table>

*Significant at the 0.05 level (2-tailed).

PCOS and non-PCOS groups, respectively. Regarding the results of HOMA-IR, profile women with PCOS (n -37, HOMA-IR value 3.0%) had more HOMA-IR values than women without PCOS (n -38.3; HOMA-IR value 2.1%) with statistically significant difference (P-0.024) (Table 1).

**5.2. Menstrual Regularity**

The main reasons for admission were infertility 38 cases due to PCOS and 23 cases with infertility for other reasons. One patient was in her menstrual phase, 39 patients (65.5%) were in the early proliferative phase and 20 patients (33.3%) were in thinned-follicular phase. Thirty patients (50.0%) had regular cycles, twenty three patients (38.3%) had oligo/amenorrhea and seven patients (11.6%) had frequent irregular cycles. Thirty-four patients (56.6%) had clinical hyperandrogenism with hirsutism and/or acne. At laparoscopy: thirty four women (56.6%) had polycystic features, one patient (1.6%) with endometriosis, one patient (1.6%) with adhesions and the rest (n-24, 40.0%) had normal findings. At hysteroscopy: fifty five cases (91.6%) had normal macroscopic findings, one case (1.6%) had blocked tubes, three cases (5.0%) had endometrial polyps and one case (1.6%) with cervical polyp. Polycystic ovary (PCO) appearance on TVS was found in 33 patients compared to 37 patients (Rotterdam Criteria) forming 89.2%. While only 3 patients without PCOS compared to 8 patients (Rotterdam Criteria) forming 37.5%.

The overall prevalence of EH was 23.3% (14 cases); including 18.3% (n-11) and 5% (n-3) in the PCOS and non-PCOS groups, respectively. Histopathological examination of patients with endometrial hyperplasia revealed eleven patients (78.6%) with simple glandular hyperplasia without atypia, and three patients (21.4%) with complex hyperplasia without atypia. Endometrial histop-
thological examination of patients with hyperplasia (n=14) revealed no cases of EH with atypia or endometrial adenocarcinoma. All participants with EH were contacted to receive appropriate treatment. On the other hand, histopathological examination also showed the following other abnormal results: one case (1.6%) with endometrial polyp, one case (1.6%) with cervical polyp, two cases (3.0%) with hormonal imbalance.

Multiple logistic regression for the entire study subjects (with and without PCOS) showed that women with endometrial thickness equal or more than 9.5 mm were about 1.28 times more at risk of developing EH than patients of the reference group (patients with endometrial thickness less than 9.5 mm). So for each one unit increase in the endometrial thickness (1 mm), there will be 28% more odds of having endometrial hyperplasia. For example for each 5 mm increase in the endometrial thickness, there will be \((1.28)^5 = 3.4\) chance (odds) to have endometrial hyperplasia (Table 2). However, the BMI and HOMA-IR was not significantly differ between EH and non-hyperplasic women.

Clinically, the fourteen women with EH were represented with the following manifestations two women (4.4%) had regular periods, nine women (20.0%) complained of oligo/amenorrhea and three women (6.7%) had irregular periods. Consequently, as illustrated in Table 2 women with oligo/amenorrhea and irregular cycles were 5.5 and 13.7 times more, respectively, at risk of developing EH than women with regular cycles.

### 5.3. Results of Participants with PCOS (n = 37)

Endometrial thickness (mm) was significantly higher in patients with hyperplasia \((t = -2.78, 0.009)\). However, patients with PCOS either with hyperplasia \((n = 11, 29.7\%)\) or without hyperplasia \((n = 26, 70.3\%)\) were not significantly different in terms of age, BMI, and insulin resistance with \(t\)-test, p-value \(-0.705, 0.485; 1.419, 0.165; -2.019, 0.051\), respectively (Table 3). In addition, endometrial thickness (mm) was positively correlated with insulin resistance \((r = 0.439, P = 0.007)\); as the insulin resistance increases there are a corresponding increase of endometrial thickness.

The only difference between the two samples of respondents was that the respondents in the hyperplasic group were significantly having higher levels of

### Table 2. Multiple logistic regressions to determine factors associated with endometrial hyperplasia in women with and without PCOS.

<table>
<thead>
<tr>
<th>Variable</th>
<th>AOR</th>
<th>95% CI</th>
<th>(\chi^2) stat. (df)*</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometrial thickness</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥9.5 mm</td>
<td>1.28</td>
<td>1.065 - 1.548</td>
<td>9.355 (1)</td>
<td>0.002</td>
</tr>
<tr>
<td>&lt;9.5 mm</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Menstrual regularity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oligo/amenorrhea</td>
<td>5.55</td>
<td>0.866 - 35.60</td>
<td>3.26\textsuperscript{b}</td>
<td>0.071\textsuperscript{bc}</td>
</tr>
<tr>
<td>Irregular</td>
<td>13.73</td>
<td>1.13 - 165.94</td>
<td>4.24\textsuperscript{b}</td>
<td>0.039\textsuperscript{b}</td>
</tr>
<tr>
<td>Regular</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

AOR = Adjusted odds ratio; \* Likelihood Ratio (LR) test; \textsuperscript{b}Wald test; \textsuperscript{c}Trend.
endometrial thickness (factor under the study) than other group (non-hyperplasic patients), as shown in Table 3.

The lowest value of the ET among women with EH was 10 mm, so we set up a 9.5 mm endometrial thickness as a cut-off value in the PCOS group. There were: twenty three patients with endometrial thickness > 9.5 mm, eleven patients of them had endometrial hyperplasia; while fourteen patients had no endometrial hyperplasia with endometrial thickness < 9.5 mm. Receiver operating characteristic curve of endometrial thickness and with the presence or absence of endometrial hyperplasia, the curve was constructed with data generated from a prospective cohort who underwent (H & E) cutoff of 9.5 mm thickness. The area under the curve was 0.738 (95% confidence interval, 0.591 - 0.886) (Figure 1). An endometrial thickness of >9.5 mm had 92.9% sensitivity and 51.85% specificity for the presence of hyperplasia (Figure 1).

In addition, women with PCOS who had EH and oligo/amenorrhea (n = 9, 40.9%) were significantly higher than those who had regular cycle (n = 2, 14.3%), this difference was statistically significant with Chi-square = 8.7, p = 0.003.

Figure 1. Receiver operating characteristic curve of endometrial thickness and presence or absence of endometrial hyperplasia, the curve was constructed with data generated from study subjects with PCOS who underwent endometrial measurement with cutoff point of 9.5 mm. Area under the curve was 0.738 (95% confidence interval, 0.591 - 0.886). An ET of >9.5 mm had 92.9% sensitivity and 54.8% Specificity for the presence of endometrial hyperplasia.
Table 3. Features of patients with PCOS (n=37).

<table>
<thead>
<tr>
<th>Features</th>
<th>t</th>
<th>P-value</th>
<th>Mean difference</th>
<th>95% CI of the difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>−0.7</td>
<td>0.485</td>
<td>−1.26</td>
<td>−4.9 to 2.4</td>
</tr>
<tr>
<td>BMI</td>
<td>1.4</td>
<td>0.165</td>
<td>0.9</td>
<td>−0.4 to 2.4</td>
</tr>
<tr>
<td>Insulin resistance</td>
<td>−2.0</td>
<td>0.051</td>
<td>−0.7</td>
<td>−1.4 to 0.0</td>
</tr>
<tr>
<td>Endometrial thickness (mm)</td>
<td>−2.8</td>
<td>0.009</td>
<td>−4.6</td>
<td>−8.0 to −1.7</td>
</tr>
</tbody>
</table>

6. Discussion

Our results showed that the prevalence of endometrial hyperplasia among infertile patients with PCOS is 18.3%. This prevalence of EH is in concordance with the figures worldwide; 1% to 48.8% [16] [24] [25]. The high prevalence of EH in our population may be explained that these are infertility cases with long standing PCOS. Sonographic endometrial thickness is predictive for endometrial hyperplasia in PCOS women [35]. We propose a new threshold value of 9.5 mm of endometrial thickness measured by vaginal U/S, which had a sensitivity of 92.9%, specificity of 51.85% positive predictive value of 47.8%, and negative predictive value of 100%. Thus, if there is a one unit increase in the endometrial thickness (1 mm), there will be a 1.26 increase in the chance of getting or developing endometrial hyperplasia in women with PCOS. Thus, for example for each 5 mm increase in the endometrial thickness, there will be (1.26)^5 = 3.2 chance (odds) to suffer from endometrial hyperplasia. The cutoff value of 9.5 mm is more practical and it achieves a good predictive value in obese and none-obese women with PCOS. Recently, McCormick et al. [24] suggested an ET of 9.35 mm as the threshold to detect endometrial hyperplasia only in obese patients with PCOS, with 100% sensitivity, 56% specificity, 100% negative predictive value, and 50% positive predictive value. In addition, another study suggested 7 mm as a threshold with 100% sensitivity but with low specificity (27.8%) for endometrial hyperplasia POCs patients [16].

Another important finding in our study was that women with oligo/amenorrhea or irregular cycles were 5.5 and 13.7 times more at risk of developing EH than women with regular cycles respectively. Another study has shown that the risk of endometrial hyperplasia raises by 43.2% (OR 1.4) in PCOS patients with amenorrhea for more than 3 - 6 months [16].

Patients with PCOS either with hyperplasia (n = 11, 29.7%) or without hyperplasia (n = 26, 70.3%) were not significantly different in terms of insulin resistance (t-test, P-value) (~2.019, 0.05). This border line correlation may be explained by the fact that all PCOS patients were on insulin sensitizing agent for the last three months. However, after controlling the effect of other covariate (menstrual phase); using partial correlation analysis; endometrial thickness (mm) was positively correlated with insulin resistance (r = 0.439, P = 0.007), which points to the importance of insulin resistance in the pathophysiology of...
PCOS and associated long term possible risk for developing EH.

Small sample size is a limitation in our study although; the sample sizes in most of literature are of approximation. However, one of the strengths of our study is that endometrial sampling was performed by curettage after a hysteroscopy which minimized the chance of missing pathology.

7. Conclusion

In summary, our findings illustrate the usefulness of obtaining a detailed menstrual history in women with PCOS especially in those with irregular periods or oligo/amenorrhea and insulin resistance in whom the endometrial thickness in vaginal U/S scan is more 9.5 mm. This valuable data will contribute to identify those having a potential risk of developing endometrial hyperplasia. Thus, we recommend that all infertile PCOS women with the above mentioned features should have an endometrial biopsy to exclude endometrial hyperplasia.

Conflicts of Interest

All Authors state no potential conflicts of interest.

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Abbreviations

ET: Endometrial Thickness
EH: Endometrial Hyperplasia
PCOS: Polycystic Ovary Syndrome
TVS: Trans-Vaginal Scan
EB: Endometrial Biopsy
HOMA-IR: Homeostasis Model Assessment-Insulin Resistance
BMI: Body Mass Index