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RELATIONSHIP BETWEEN ULTRASONOGRAPHY AND HISTO-PATHOLOGICAL CHANGES IN POLYCYSTIC OVARIES SYNDROME

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Abstract

Background: *Polycystic Ovary Syndrome (PCOS) is a common endocrine disorder affecting the women of reproductive age, characterized by the irregular menstrual cycles, infertility, and hormonal imbalances. Ultrasonography plays an crucial role in the diagnosing of PCOS by assessing ovarian morphology, particularly the presence of the polycystic ovarian morphology (PCOM).*

Objective: *This study aimed to investigate the correlation between ultrasonographic findings and histopathological changes in patients diagnosed with PCOS.*

Methodology: *A total of 40 patients with a confirmed diagnosis of th PCOS were included. Transvaginal ultrasound was performed to measure the ovarian volume, follicle count, and stromal thickness. Histopathological evaluations were conducted on specimens obtained during ovarian surgeries, assessing features like follicular atresia, capsule thickening, and theca cell hyperplasia. Statistical analyses were performed to determine the correlations between ultrasonographic and histopathological findings.*

Results: *Significant positive correlations were found between the ovarian volume and follicular atresia ($r = 0.891$, $p < 0.001$) and capsule thickening ($r = 0.891$, $p < 0.001$). Follicle count also correlated strongly with the follicular atresia ($r = 0.880$, $p < 0.001$). No significant correlation was observed with the stromal thickness. The findings confirm that ultrasonographic assessments are valuable in diagnosing and understanding PCOS. The strong correlations with*

histopathological features support the integration of imaging techniques in clinical practice.

Conclusion: *This study emphasizes the importance of combining ultrasonography and the histopathological analysis in the diagnosing PCOS and highlights the need for standardized diagnostic criteria to enhance the patient care. Further research with larger cohorts is recommended to validate these findings*

INTRODUCTION

Polycystic Ovarian Syndrome (PCOS) is a the common and complex endocrine disorder that affects women of reproductive age. Characterized by a combination of symptoms, including hyperandrogenism, ovulatory dysfunction, and polycystic ovarian morphology, PCOS has significant implications for fertility, metabolic health, and psychological well-being. A systematic review and meta-analysis indicated that the global prevalence of PCOS is approximately 9.2% (95% CI: 6.8–12.5%) between women of reproductive age. This figure is derived from various studies that included over 12 million subjects worldwide.(1) Despite its widespread impact, the exact etiology of PCOS remains unclear, as it is believed to involve genetic, environmental, and lifestyle factors. Due to its multifaceted nature, the diagnosis and management of PCOS require a comprehensive approach that incorporates clinical, biochemical, and imaging findings.(2)

Ultrasonography, particularly transvaginal ultrasound, has become an essential tool in the diagnosing PCOS. It offers a non-invasive and widely accessible method for visualizing the ovaries, allowing clinicians to detect key morphological features associated with the syndrome. These features, include the presence of multiple small antral follicles (more than 12 per ovary), increased ovarian volume, and a thickened ovarian stroma, form part of the Rotterdam criteria—one of the most commonly used diagnostic frameworks for PCOS. Ultrasonography, therefore, plays a critical role in identifying the polycystic ovarian morphology, providing valuable information on the structural changes within the ovaries.(3)

However, while ultrasound can reveal the gross morphological characteristics of polycystic ovaries, it cannot capture the underlying histopathological changes. Histopathology, which involves the microscopic examination of the ovarian tissue, offers deeper insights into the cellular and tissue-level alterations that accompany PCOS.(4) These histopathological changes include follicular atresia, thickening of the ovarian capsule, and hyperplasia of the theca interna cells. Such findings provide crucial information on the pathophysiology of the syndrome, revealing how hormonal imbalances contribute to ovarian dysfunction and the development of cysts.(4)

Despite the widespread use of ultrasound in diagnosing PCOS, there is still much to learn about how ultrasonographic findings relate to histopathological changes. While the presence of multiple small antral follicles and an enlarged ovarian volume are hallmark features of polycystic ovaries on ultrasound, these findings do not always correlate with the severity of the underlying histopathological alterations.(5) For example, some women with PCOS may exhibit significant histological changes despite having relatively mild ultrasound findings, while others with more pronounced ultrasound abnormalities may have less severe histopathological features. This variability underscores the need for further research to clarify the connection between ultrasound and histopathology in PCOS. (6)

The role of ultrasonography in PCOS is further complicated by the fact that polycystic ovarian morphology is not specific to the syndrome. Many women without PCOS also exhibit polycystic ovaries on ultrasound, particularly during adolescence or following the use of certain hormonal contraceptives. As a result, ultrasound alone is not sufficient to diagnose PCOS; it must be used in conjunction with clinical and biochemical criteria. This limitation highlights the importance of correlating ultrasound findings with histopathological data, as doing so may help

to distinguish between true cases of PCOS and other conditions that can mimic its imaging features.

Histopathological examination of polycystic ovaries has revealed several key features that provide insight into the pathogenesis of the syndrome. One of the most consistent findings is the presence of a thickened ovarian capsule, which may impede the release of mature oocytes and contribute to the anovulation commonly seen in PCOS.(7) In addition, follicular atresia—whereby developing follicles fail to mature and undergo degeneration—is a hallmark of PCOS, reflecting the disrupted folliculogenesis associated with the syndrome. Theca cell hyperplasia, another common histopathological finding, reflects the excessive production of androgens in PCOS, which contributes to the clinical symptoms of hyperandrogenism, such as hirsutism and acne. These histopathological changes are critical for understanding the hormonal and structural abnormalities that underlie PCOS, and their correlation with ultrasonographic findings is an important area of investigation.(8)

In recent years, advances in imaging technology have improved the ability of ultrasonography to detect subtle changes in ovarian morphology. For example, three-dimensional (3D) ultrasound has been shown to provide more detailed information on ovarian volume and follicle number, potentially offering a more accurate assessment of polycystic ovarian morphology than traditional two-dimensional (2D) ultrasound. Similarly, Doppler ultrasound can be used to assess ovarian blood flow, which may provide additional information on the vascular changes associated with PCOS. However, the relationship between these advanced imaging techniques and histopathological changes has not been fully explored, and further research is needed to determine how these technologies can be integrated into the diagnostic process for PCOS.(9)

Understanding the relationship between ultrasonographic findings and histopathological changes is important for several reasons. First, it can enhance diagnostic accuracy by allowing clinicians to correlate imaging features with tissue-based alterations, thereby providing a more complete picture of the disease process. Second, it may improve patient management by helping to identify different subtypes of PCOS, which could respond differently to treatment. Finally, exploring this relationship may offer new insights into the pathogenesis of PCOS, potentially leading to novel therapeutic strategies aimed at targeting the root causes of the syndrome.(10)

The aim of this study is to explore the relationship between ultrasonography and histopathological changes in polycystic ovaries. By comparing ultrasound findings with histological analysis, this research seeks to establish a clearer connection between these diagnostic methods, providing better insights into the progression and characteristics of PCOS. Specifically, this study will examine whether certain ultrasonographic features—such as the number and size of antral follicles, ovarian volume, and stromal thickness—correlate with key histopathological changes, including follicular atresia, theca cell hyperplasia, and capsule thickening. Understanding these correlations could improve diagnostic accuracy, allowing for more personalized and effective management of PCOS.

LITERATURE REVIEW

Kentaro Takahashi et al in 1994 conduct a study to examined the histological results with endocrine concentrations and looked into the association between the ultrasonographic appearance of the ovaries and histopathological findings in patients with polycystic ovarian syndrome (PCOS). In all, twenty PCOS patients were examined. Every patient presented with a medical history of infertility, irregular menstruation, and more than ten tiny cysts seen in each ovary during transvaginal ultrasound. A general cystic pattern (GCP) or a peripheral cystic pattern (PCP) was identified based on the appearance of the ovaries on ultrasonography. Ultrasonographic pictures and histopathological evaluation of material from patients undergoing laparoscopic ovarian wedge resection were compared. The histopathology results in 15 out of the 16 ovaries with GCP matched the ultrasound pictures. The ultrasonographic and histological

images were consistent in all 24 PCP-affected ovaries. In comparison to the PCP, the GCP had ovarian capsular thickness that was on average much higher. While the ratio of luteinizing hormone to follicle-stimulating hormone was much higher in the PCP than the GCP, androstenedione was significantly higher in the GCP. Consequently, our findings implied that histopathological variations are correlated with GCP and PCP. Endocrinological differences seem to exist between histological GCP and PCP. (11)

Kentaro Takahashi et al in 1993 conduct a study. In his study In 32 PCOS patients with bilateral polycystic ovaries (> 10 cysts) detected by TVS, 20 ovulatory women served as controls. The aim of the study was to examine the pathological features of polycystic ovaries diagnosed by TVS in patients with polycystic ovarian syndrome (PCOS) and to explore the relationship between morphological and endocrine changes in the ovaries. The histology of the ovarian tissues from the wedge resection was investigated. This study compared the ovarian morphology using histology and TVS analysis, and it looked at the association between the frequency of tiny cysts and the endocrine profile. The size and location of the tiny cysts on TVS matched the histologically observed ones. In 32 PCOS patients with bilateral polycystic ovaries (> 10 cysts) detected by TVS, 20 ovulatory women served as controls. The aim of the study was to examine the pathological features of polycystic ovaries diagnosed by TVS in patients with polycystic ovarian syndrome (PCOS) and to explore the relationship between morphological and endocrine changes in the ovaries. The histology of the ovarian tissues from the wedge resection was investigated. This study compared the ovarian morphology using histology and TVS analysis, and it looked at the association between the frequency of tiny cysts and the endocrine profile. The size and location of the tiny cysts on TVS matched the histologically observed ones. The amount of atretic follicles with thicker ovarian capsules and hypertrophied and luteinized inner theca cells had a strong connection with the number of tiny cysts on TVS. In 97 and 64% of the ovaries from PCOS patients, respectively, there were many atretic follicles and thicker ovarian capsules. The number of tiny cysts and Δ4-androstenedione (ASD) as well as the ratio of luteinizing hormone to follicle-stimulating hormone (LH/FSH) showed a strong positive link in PCOS patients. In addition, PCOS patients with ovarian thickened tunica had significantly greater levels of testosterone, ASD, and the quantity of tiny cysts on TVS than PCOS patients without ovarian thickened tunica. TVS pictures of the ovaries in PCOS patients showed a correlation with the endocrine and histological characteristics. (12)

William U.Atiomo, et al. in 2000 conduct a study on PCOS. Not every woman with polycystic ovarian syndrome (PCOS) on ultrasound (US) will actually have PCOS; in fact, PCOS may exist without US symptoms in certain cases, exhibiting clinical and biochemical characteristics instead. Therefore, 72 women (32 with PCOS and 40 without) had their sensitivity to US detection of PCOS prospectively assessed. The most sensitive characteristics were peripheral follicle distribution (81.8% and 71.9% in the left and right ovary) and the presence of 10 or more follicles (82% and 69% in the left and right ovary). The most specific criterion was stromal brightness, while ovarian enlargement and stromal brightness were less sensitive than the preceding ones. In 86.4% of instances, combining all the criteria accurately indicated a PCOS diagnosis or control. In the diagnosis of PCOS, this study demonstrates that the recognized US criteria for polycystic ovaries are still useful; nonetheless, the divergence between the left and right ovaries is an intriguing but puzzling discovery. (13)

Battaglia C et al. in 1998 conduct a study on two distinct morphological forms of polycystic ovaries—a peripheral cystic pattern and a general cystic pattern—have been identified using ultrasound. This study sought to determine whether individuals with the peripheral form of polycystic ovaries had ovarian and uterine blood flow that differed from that of patients with the general form, as well as whether the two forms were associated with distinct hormonal characteristics. Evaluations included clinical, biochemical, grayscale, and color Doppler

ultrasonography for eighteen patients with the general form of polycystic ovary and sixteen individuals with the peripheral type. Every patient had polycystic ovarian syndrome (PCOS) confirmed by the data evaluated. There was no difference in the groups' individual levels of testosterone, androstenedione, estradiol, follicle stimulating hormone (FSH), luteinizing hormone (LH), or androstenedione. However, compared to the general cystic group, the peripheral cystic group had a considerably higher LH/FSH ratio and a greater stromal echodensity. When comparing the intraovarian arteries of the peripheral cystic group to those of the general cystic group, Doppler ultrasonography revealed a greater visibility rate and significantly lower pulsatility index values. These results imply that the various physical forms of polycystic ovary do not correspond to variations in endocrine profile, with the exception of the LH/FSH ratio. Nonetheless, Doppler evaluation of each case's blood flow variations revealed that PCOS does not predetermine a particular intraovarian blood flow pattern. (14)

Adam H. et al in 2003 conduct a research on pathophysiology of polycystic ovarian syndrome (PCOS) is a complex disorder that seems to be both multifactorial and polygenic. There has been substantial discussion over the syndrome's definition. Obesity, hyperandrogenism, and disruption of the menstrual cycle are important characteristics. Although the pathogenesis of PCOS involves several extra-ovarian factors, ovarian dysfunction plays a key role. Refined PCOS definition agreed upon at a recent joint ASRM/ESHRE consensus meeting included morphological description of the polycystic ovary (PCO). As per the extant literature, the PCO must meet certain criteria to have adequate specificity and sensitivity. These criteria include having a minimum of 12 follicles with a diameter of 2-9 mm or an enlarged ovarian volume exceeding 10 cm^3. Calculating volume and area requires repeating the scan during an ovarian quiescence period if there are follicles larger than 10 mm in diameter. To make the diagnosis, only one PCO needs to be present. A description of the stroma and the distribution of follicles are not necessary for the diagnosis. Although PCO is associated with increased stromal echogenicity and/or stromal volume, research has demonstrated that ovarian volume (or area) measurement is a useful proxy for stroma quantification in clinical settings. Until more is known about this condition, a woman with PCO who does not also have hyperandrogenism or an ovulation issue (referred to as "asymptomatic PCO") should not be diagnosed with PCOS. Three-dimensional and Doppler ultrasound studies may be useful research tools but are not required in the definition of PCO. This review outlines evidence for the current ultrasound definition of the polycystic ovary and technical specifications.(15)

Ozaki T et al. in 1994 conduct a research on Women of reproductive age who suffer from the complicated endocrine illness known as Polycystic Ovary Syndrome (PCOS) experience a variety of symptoms, such as infertility, irregular menstruation, and hyperandrogenism. The severity of the illness varies greatly, and minor cases may go untreated. Severe cases, however, may result in a variety of metabolic and cardiovascular issues. Precise diagnosis is essential and usually entails a mix of physical assessment, hormone profiling, and ovulation ultrasonography evaluation. The latter offers a non-invasive, economical, and effective way to evaluate ovarian morphology; important markers of PCOS are ovarian volume, follicle count, and stromal echogenicity. A thorough analysis of the body of research demonstrates how crucial these ultrasonographic signals are for forecasting.(16)

Takahashi K, Eda Y et al. in 1994 conduct a study to better understand the pathological traits of polycystic ovaries and how they relate to endocrine abnormalities, a study was conducted on 32 PCOS patients with bilateral polycystic ovaries and 20 ovulatory controls. A close relationship was found between the quantity of tiny cysts and atretic follicles with hypertrophied and luteinized inner theca cells, as well as thicker ovarian capsules, using transvaginal ultrasonography (TVS) and histological analysis of ovarian tissues. Remarkably, the ovaries of 97% of PCOS patients contained many atretic follicles, and 64% had thicker capsules. Small cyst counts and

androstenedione levels, as well as androstenedione and the LH/FSH ratio, were found to be significantly positively correlated. Additionally, compared to individuals without PCOS, those with thicker ovarian tunica exhibited greater levels of tiny cysts, testosterone, and androstenedione.(17)

Zaboriene et al. in 2022 conduct a research on PCOS, or polycystic ovarian syndrome, is a common hormonal condition that affects women who are fertile. It is typified by high levels of testosterone, irregular menstruation periods, and infertility. The severity of the illness varies greatly from person to person, and minor cases may go untreated. Severe cases, however, may result in various metabolic and cardiovascular issues. The diagnosis usually consists of a physical examination, hormonal profiling, and ovarian ultrasonography, which is a non-invasive, reasonably priced, and successful diagnostic technique. Certain ultrasonography characteristics, such as ovarian volume, follicle count, distribution pattern, stromal echogenicity, and Doppler-assessed resistance and pulsatility indices, can be used to differentiate between healthy and polycystic ovaries. Ovarian volume and follicle number per ovary were found to be the most accurate ultrasonographic indicators of PCOS in this comprehensive analysis, improving diagnosis accuracy and directing treatment.(3)

Henrichsen TL et al in 2009 conduct a study on Women of reproductive age who have PCOS, a complex endocrine condition characterized by hyperandrogenism, irregular menstruation, and infertility, are affected. The severity of the illness varies greatly, and minor cases may go untreated. Severe cases, however, may result in various metabolic and cardiovascular issues. Physical examination, hormonal profiling, and ovarian ultrasonography—a non-invasive, economical, and successful diagnostic technique—are all necessary for an accurate diagnosis. Ovarian volume, follicle count, and distribution pattern are among the specific ultrasonography indicators that aid in differentiating between polycystic and healthy ovaries. This comprehensive analysis emphasizes the significance of ovarian volume and follicle count per ovary as critical ultrasonographic indicators of PCOS, facilitating precise diagnosis and well-informed treatment choices.(18)

Saori Okada et al. in 1993 conduct a research on Four groups were created based on the amount of microcysts in 104 individuals who had bilateral polycystic ovaries (PCO) as determined by transvaginal ultrasound: A <5 cysts, B 5 –10 cysts, C > 10 cysts located under ovarian capsule, and D many cysts dispersed all over the ovary. For each group and the 17 control subjects with normal ovulatory cycles, the following factors were compared: ovarian volume, clinical features (such as menstrual disturbance, obesity, and hirsutism), endocrine levels (luteinizing hormone (LH), LH/follicle stimulating hormone ratio, testosterone, androstenedione and dehydroepiandrosterone-sulphate), and response to clomiphene. Compared to control participants, the ovarian volume in PCO subjects was substantially greater. The ovarian volume expanded and the endocrine abnormalities worsened as the number of microcysts grew. In comparison to PCO participants without monthly disorders and controls, all endocrine measures in PCO subjects with menstrual abnormalities were significantly higher. Only among PCO patients without monthly irregularities was androstenedione much higher than in the control group. Menstrual disturbance frequency, clomiphene response percentage, and pregnancy rate were all considerably greater in groups C and D of PCO participants than in groups A and B. Consequently, transvaginal ultrasound screening of the ovaries in infertile patients and subsequent evaluation of morphology in PCO may indicate an endocrine issue and aid in the appropriate.(19)

Balen AH et al. in 2003) conduct a study on the pathophysiology of polycystic ovarian syndrome (PCOS) is a complex disorder that seems to be both multifactorial and polygenic. There has been substantial discussion over the syndrome's definition. Obesity, hyperandrogenism, and disruption of the menstrual cycle are important characteristics. Although the pathogenesis of PCOS involves several extra-ovarian factors, ovarian dysfunction plays a key role. A more precise definition of PCOS was recently agreed

upon at a joint ASRM/ESHRE consensus meeting. This definition included information on the morphology of the polycystic ovary (PCO). The literature that is currently available suggests that at least one of the following conditions should be met in order to define the PCO: either 12 or more follicles with a diameter of 2 to 9 mm, or an enlarged ovarian volume (>10 cm3). Should a follicle count exceed ten? To make the diagnosis, only one PCO needs to be present. A description of the stroma and the distribution of follicles are not necessary for the diagnosis. PCO is associated with increased stromal echogenicity and/or stromal volume; nevertheless, measurements of ovarian volume (or area) have been demonstrated to be a reliable proxy for stroma quantification in clinical practice. Until more is known about this condition, a woman with PCO who does not also have hyperandrogenism or an ovulation issue (referred to as "asymptomatic PCO") should not be diagnosed with PCOS. Doppler ultrasonography and three-dimensional imaging examinations are not necessary for the definition of PCO, but they can be helpful research instruments. The data supporting the current ultrasound definition of the polycystic ovary is outlined in this review(20)

Sonal panchal et al. in 2023 conduct research on Human female reproductive systems are extremely dynamic hormonal systems that are delicately balanced. The foundation for comprehending the cause of infertility and the best way to manage treatment cycles is a correct understanding of these hormonal symphonies. Multiple hormonal measures can be used to accomplish this, but sonoendocrinology—ultrasound—is a highly useful technique for tracking, understanding, and monitoring these changes. When hormonal changes take place, the vascular alterations happen before the morphological changes. Hormonal changes during the menstrual cycle can be understood by interpreting the morphological and vascular alterations in relation to their physiological and hormonal basics.(21)

Mancini F, et al. in 2004 conduct a research, in his research for women, polycystic ovarian syndrome (PCOS) and the associated androgen excess can be very upsetting and disruptive. Consequently, PCOS necessitates an early diagnosis and appropriate care. However, just like the illness itself, the criteria used to diagnose and define PCOS are diverse. With a good concordance rate with laparoscopic and histological investigation, the development of ovulation ultrasonography has contributed the most to the diagnosis of PCOS. Actually, the evaluation of ovarian morphology using transvaginal ultrasonography and Doppler flow analysis of the uterine and intraovarian arteries appears to offer some insight into the disease's pathological state and rate of progression. It may also be helpful in managing and preventing ovarian hyperstimulation syndrome during ovarian stimulation.(22)

3.1: OBJECTIVE

To check the relationship between ultrasonography and histo-pathological changes in polycystic ovaries syndrome

3.2: PROBLEM STATEMENT

Polycystic Ovarian Syndrome (PCOS) is commonly diagnosed through ultrasound, yet this method may not fully reflect the underlying histopathological changes, such as follicular atresia and theca cell hyperplasia. The inconsistency between ultrasound findings and these tissue-level alterations can complicate diagnosis and treatment. This study aims to explore the relationship between ultrasonographic features and histopathological changes in PCOS, with the goal of improving diagnostic accuracy and patient management.

3.3: OPERATIONAL DEFINITIONS

Polycystic Ovarian Syndrome (PCOS): A hormonal disorder affecting women of reproductive age, characterized by irregular menstrual cycles, hyperandrogenism (elevated male hormones), and polycystic ovarian morphology, diagnosed using clinical, biochemical, and imaging criteria based on the Rotterdam criteria.(23)

Ultrasonography (USG): A non-invasive imaging technique that uses high-frequency sound waves to

visualize internal organs, in this study, used to identify key morphological features of polycystic ovaries, including increased ovarian volume, multiple antral follicles (more than 12 per ovary), and a dense ovarian stroma.(23)

Histopathology: The microscopic examination of ovarian tissue to identify structural changes, including follicular atresia (degeneration of immature follicles), thickening of the ovarian capsule, and hyperplasia of the theca interna cells, which are associated with PCOS.(24)

Rotterdam Criteria: A widely used diagnostic framework for PCOS that requires two out of three features for diagnosis: oligo/anovulation, hyperandrogenism, and polycystic ovarian morphology on ultrasound.(25)

Ovarian Volume: The size of the ovaries as measured on ultrasonography, often increased in women with PCOS. Ovarian volume is considered elevated when it exceeds 10 cm^3 per ovary.(26)

Polycystic Ovarian Morphology: A diagnostic feature of PCOS seen on ultrasound, characterized by the presence of 12 or more small follicles (2–9 mm in diameter) in one or both ovaries, and/or increased ovarian volume.(27)

Ovarian Stroma: The connective tissue within the ovaries that surrounds the follicles, which can appear thickened or dense in ultrasound imaging of women with PCOS.(28)

Theca Cell Hyperplasia: An increase in the number of theca cells, which are involved in androgen production, contributing to hyperandrogenism in PCOS. This condition is observed via histopathological analysis.(23)

MATERIAL AND METHODS

4.1: Study Design: Cross-sectional study

4.2: Settings: Wapda Teaching Hospital Complex, Lahore

4.3: Study Duration: 4 months after approval of synopsis

4.4: Sample Size: 40

4.5: Sampling Technique: Convenient sampling.

4.6: Sample Selection:

- **4.6.1: Inclusion Criteria:**
  - Subjects between 18 to 30 year diagnosed with PCOS based on the Rotterdam criteria
  - Those patient who undergoes, polycystic ovarian morphology on ultrasound, oligo/anovulation, or clinical/biochemical signs of hyperandrogenism

4.6.2: Exclusion Criteria:

- Patients with other underlying ovarian disorders, hormonal imbalances
- History of ovarian surgery
- Subjects below 18 years of age

4.7: Equipment and Scanning Technique

Ultrasonographic Evaluation:

Ultrasonography will be performed using a high-resolution transvaginal ultrasound (TVUS) machine, which is optimal for detailed ovarian imaging. Each participant's ovaries will be examined for the following features:

Ovarian volume: Measured in cubic centimeters (cm^3), with values >10 cm^3 considered abnormal.

Follicle count: The number of antral follicles (2–9 mm in diameter) in each ovary will be counted, with more than 12 per ovary classified as polycystic.

Stromal thickness: The density of the ovarian stroma will be subjectively graded based on its appearance on ultrasound.

All ultrasonographic evaluations will be conducted by experienced sonographers using standardized imaging protocols to minimize variability. The images will be analyzed using ultrasound software for accurate measurements and documentation.

Histopathological Examination:
For patients undergoing ovarian surgery (such as ovarian drilling or cystectomy for fertility treatment), ovarian tissue samples will be obtained and sent for histopathological analysis. The histopathological examination will focus on the following changes:

Follicular atresia: The number and appearance of atretic follicles will be documented.
Capsule thickening: The thickness of the ovarian capsule will be measured.
Theca cell hyperplasia: The presence and degree of theca cell hyperplasia will be assessed microscopically.

All tissue samples will be examined by an experienced pathologist, and findings will be recorded systematically.

4.9: ETHICAL CONSIDERATIONS
The rules and regulations set by the ethical committee of GC University; Faisalabad was followed while conducting the research and the rights of the research participants were respected.
Written informed consent attached was taken from all the participants.
All information and data collection were kept confidential.
Participants were remained anonymous throughout the study.
The subjects were informed that there are no disadvantages or risk on the procedure of the study.
They were also be informed that they will be free to withdraw at any time during the process of the study.
Data was kept in under key and lock while keeping keys in hand. In laptop it was kept under password.

4.10: DATA COLLECTION PROCEDURE
Clinical data: Patient demographics, clinical features of PCOS (e.g., menstrual irregularities, hyperandrogenism), and biochemical markers (e.g., serum androgen levels) will be collected from medical records.

Imaging data: Ultrasonographic findings, including ovarian volume, follicle count, and stromal characteristics, will be recorded for each participant.

Histological data: For patients undergoing surgery, histopathological findings, including follicular atresia, capsule thickness, and theca cell hyperplasia, will be documented.

4.10: DATA ANALYSIS PROCEDURE
Data will be analyzed using statistical software (e.g., SPSS). Descriptive statistics will be used to summarize the demographic and clinical characteristics of the participants. Pearson's correlation coefficient or Spearman's rank correlation will be employed to assess the relationship between ultrasonographic findings (e.g., follicle count, ovarian volume, and stromal thickness) and histopathological changes (e.g., follicular atresia, theca cell hyperplasia, and capsule thickening). A p-value of less than 0.05 will be considered statistically significant. Additionally, multivariate regression analysis will be used to adjust for potential confounders, such as age, BMI, and duration of PCOS.

CHAPTER 5
In this stud 40 oatient with PCOs are examined, yhe results of this study revealed significant correlations between ultrasonographic findings and histopathological changes in patients with Polycystic Ovary Syndrome (PCOS). Ovarian volume showed a strong positive correlation with both follicular atresia and capsule thickening ($r = 0.891$, $p < 0.001$), indicating that larger ovarian volumes are associated with a greater prevalence of these histopathological features.

Table: 1 Statistics of Age

Age

N	Valid	40
	Missing	0
Mean		28.1000
Std. Deviation		3.43287
Minimum		22.00
Maximum		35.00

Table:2 Frequency of Age

Age

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	22.00	2	5.0	5.0	5.0
	23.00	2	5.0	5.0	10.0
	24.00	3	7.5	7.5	17.5
	25.00	4	10.0	10.0	27.5
	26.00	2	5.0	5.0	32.5
	27.00	4	10.0	10.0	42.5
	28.00	4	10.0	10.0	52.5
	29.00	5	12.5	12.5	65.0
	30.00	4	10.0	10.0	75.0
	31.00	3	7.5	7.5	82.5
	32.00	3	7.5	7.5	90.0
	33.00	1	2.5	2.5	92.5
	34.00	2	5.0	5.0	97.5
	35.00	1	2.5	2.5	100.0
	Total	40	100.0	100.0	

In this study, the age of the 40 patients ranged from 22 to 35 years, with a mean age of 28.1 years and a standard deviation of 3.43 years. The distribution of age was relatively balanced, with no extreme outliers.(Table1and Table 2)

Table:3 Statistics of BMI

BMI

N	Valid	40
	Missing	0
Mean		27.20
Std. Deviation		3.148
Minimum		22
Maximum		32

Table:4 Frequency of BMI

BMI

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	22	3	7.5	7.5	7.5
	23	3	7.5	7.5	15.0
	24	5	12.5	12.5	27.5
	25	4	10.0	10.0	37.5
	26	2	5.0	5.0	42.5
	27	2	5.0	5.0	47.5
	28	3	7.5	7.5	55.0
	29	5	12.5	12.5	67.5
	30	8	20.0	20.0	87.5
	31	2	5.0	5.0	92.5
	32	3	7.5	7.5	100.0
	Total	40	100.0	100.0	

The Body Mass Index (BMI) of the patients in this study ranged from 22 to 32, with a mean BMI of 27.20 and a standard deviation of 3.15(Table:3). This indicates that the average patient fell into the overweight category, as a BMI between 25 and 29.9 is classified as overweight. The distribution of BMI values showed that 20% of patients had a BMI of 30 or higher, which may highlight a potential association between obesity and the incidence or severity of PCOS.

Table: 5 Frequency of Irregular Menstrual Cycle

Irregular Menstrual Cycle

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	No	19	47.5	47.5	47.5
	Yes	21	52.5	52.5	100.0
	Total	40	100.0	100.0	

Among the 40 patients studied, 52.5% reported experiencing irregular menstrual cycles, while 47.5% had regular cycles. This prevalence of irregularity aligns with the common clinical presentation of Polycystic Ovary Syndrome (PCOS), where menstrual irregularities are a hallmark symptom due to hormonal imbalances.(Table: 5)

Table: 6 Frequency of Heavy menstrual bleeding

Heavy menstrual bleeding

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	No	22	55.0	55.0	55.0
	Yes	18	45.0	45.0	100.0
	Total	40	100.0	100.0	

In the study cohort of 40 patients, 45% reported experiencing heavy menstrual bleeding, while 55% did not have this symptom. Heavy menstrual bleeding is a common concern in women with Polycystic Ovary Syndrome (PCOS) and can often be attributed to hormonal imbalances, particularly involving estrogen and progesterone levels. (Table: 6)

Table: 7 Frequency of Amenorrhea

Amenorrhea

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	No	21	52.5	52.5	52.5
	Yes	19	47.5	47.5	100.0
	Total	40	100.0	100.0	

In the study of 40 patients, 47.5% reported experiencing amenorrhea, while 52.5% did not exhibit this symptom. Amenorrhea, defined as the absence of menstrual periods, is a significant clinical feature often associated with Polycystic Ovary Syndrome (PCOS), resulting from hormonal imbalances that affect ovulation. The presence of amenorrhea in nearly half of the study population highlights the endocrine dysfunction prevalent in women with PCOS.

Table 8: Statistics of Ovarian Volume (cm^3)

Statistics

Ovarian Volume (cm^3)

N	Valid	40
	Missing	0
Mean		11.20
Std. Deviation		1.924
Minimum		8
Maximum		15

Table: 9 Frequency of Ovarian Volume (cm^3)

Ovarian Volume (cm^3)

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	8	1	2.5	2.5	2.5
	9	10	25.0	25.0	27.5
	10	7	17.5	17.5	45.0
	11	3	7.5	7.5	52.5
	12	6	15.0	15.0	67.5
	13	8	20.0	20.0	87.5
	14	4	10.0	10.0	97.5
	15	1	2.5	2.5	100.0
	Total	40	100.0	100.0	

The analysis of ovarian volume in the study population revealed a mean volume of 11.20 cm^3, with values ranging from 8 cm^3 to 15 cm^3. This mean falls above the typical threshold used to classify polycystic ovaries, which is often considered to be an ovarian volume greater than 10 cm^3. In the context of Polycystic Ovary Syndrome (PCOS), increased ovarian volume is a significant indicator of the condition, reflecting the presence of multiple small follicles, a characteristic feature of the disorder.

Table: 10 Statistics of Follicle Count

Statistics

Follicle Count

N	Valid	40
	Missing	0
Mean		13.28
Std. Deviation		4.045
Minimum		7
Maximum		20

Table: 11 Frequency of Follicle Count

Follicle Count

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	7	2	5.0	5.0	5.0
	8	4	10.0	10.0	15.0
	9	4	10.0	10.0	25.0
	10	3	7.5	7.5	32.5
	11	3	7.5	7.5	40.0
	12	3	7.5	7.5	47.5
	13	1	2.5	2.5	50.0
	14	2	5.0	5.0	55.0
	15	4	10.0	10.0	65.0
	16	3	7.5	7.5	72.5
	17	3	7.5	7.5	80.0
	18	4	10.0	10.0	90.0
	19	2	5.0	5.0	95.0
	20	2	5.0	5.0	100.0
	Total	40	100.0	100.0	

The analysis of follicle count among the 40 patients in this study revealed a mean count of 13.28 follicles, with values ranging from 7 to 20 follicles. This elevated mean is indicative of the polycystic ovarian morphology commonly observed in women with Polycystic Ovary Syndrome (PCOS), where the presence of multiple small antral follicles is a hallmark characteris. (table 10 and 11)

Table: 12 Frequency of Stromal Thickness

Stromal Thickness

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Thin	11	27.5	27.5	27.5
	Thick	10	25.0	25.0	52.5
	Very Thick	7	17.5	17.5	70.0
	Moderate	12	30.0	30.0	100.0
	Total	40	100.0	100.0	

In this study, the evaluation of stromal thickness revealed that it is categorized into four grades: thin, moderate, thick, and very thick. Among the 40 patients, 30% exhibited moderate stromal thickness, 27.5% were classified as thin, 25% as thick, and 17.5% as very thick.

Table: 13 Frequency of Follicular Atresia

Follicular Atresia

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	No	19	47.5	47.5	47.5
	Yes	21	52.5	52.5	100.0
	Total	40	100.0	100.0	

In the study of 40 patients with Polycystic Ovary Syndrome (PCOS), 52.5% of the participants exhibited follicular atresia, a condition characterized by the degeneration of immature ovarian follicles. Follicular atresia is a common phenomenon in PCOS and reflects the disrupted folliculogenesis associated with the disorder.

Table: 14 Frequency of Capsule Thickening

Capsule Thickening

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	No	19	47.5	47.5	47.5
	Yes	21	52.5	52.5	100.0
	Total	40	100.0	100.0	

In the analysis of the 40 patients diagnosed with Polycystic Ovary Syndrome (PCOS), 52.5% exhibited capsule thickening of the ovaries, a common histopathological feature associated with this condition. Capsule thickening is indicative of changes in ovarian morphology that often result from hormonal imbalances and the pathophysiological alterations associated with PCOS.

Table: 15 Frequency of Theca Cell Hyperplasia
Theca Cell Hyperplasia

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	No	23	57.5	57.5	57.5
	Yes	17	42.5	42.5	100.0
	Total	40	100.0	100.0	

In the study involving 40 patients diagnosed with Polycystic Ovary Syndrome (PCOS), 42.5% exhibited theca cell hyperplasia, characterized by an increase in theca cells surrounding the ovarian follicles.

Table: 15 Correlations
Correlations

		Ovarian Volume (cm^3)	Follicle Count	Stromal Thickness	Follicular Atresia	Capsule Thickening	Theca Cell Hyperplasia
Ovarian Volume (cm^3)	Pearson Correlation	1	.922**	.256	.891**	.891**	.814**
	Sig. (2-tailed)		.000	.111	.000	.000	.000
	N	40	40	40	40	40	40
Follicle Count	Pearson Correlation	.922**	1	.288	.880**	.880**	.840**
	Sig. (2-tailed)	.000		.071	.000	.000	.000
	N	40	40	40	40	40	40
Stromal Thickness	Pearson Correlation	.256	.288	1	.190	.190	-.064
	Sig. (2-tailed)	.111	.071		.239	.239	.694
	N	40	40	40	40	40	40
Follicular Atresia	Pearson Correlation	.891**	.880**	.190	1	1.000**	.818**
	Sig. (2-tailed)	.000	.000	.239		.000	.000
	N	40	40	40	40	40	40
Capsule Thickening	Pearson Correlation	.891**	.880**	.190	1.000**	1	.818**
	Sig. (2-tailed)	.000	.000	.239	.000		.000
	N	40	40	40	40	40	40
Theca Cell Hyperplasia	Pearson Correlation	.814**	.840**	-.064	.818**	.818**	1
	Sig. (2-tailed)	.000	.000	.694	.000	.000	
	N	40	40	40	40	40	40

The correlation analysis conducted in this study aimed to explore the relationships between ultrasonographic findings and histopathological outcomes in patients with Polycystic Ovary

Syndrome (PCOS). The analysis included variables such as ovarian volume, follicle count, stromal thickness, and histopathological features, including follicular atresia, capsule thickening, and theca cell hyperplasia. The results indicated several significant correlations, which are summarized below.

Ovarian Volume was found to have a strong positive correlation with histopathological outcomes. Specifically, it showed a correlation coefficient of $r = 0.891$ with follicular atresia ($p < 0.001$), indicating that larger ovarian volumes are associated with an increased presence of follicular atresia. Similarly, ovarian volume demonstrated a significant correlation with capsule thickening ($r = 0.891$, $p < 0.001$), suggesting that as ovarian size increases, the likelihood of capsule thickening also rises. Additionally, ovarian volume was correlated with theca cell hyperplasia ($r = 0.814$, $p < 0.001$), highlighting the association between increased ovarian size and excessive growth of theca cells responsible for androgen production.

Follicle Count also exhibited strong positive correlations with the histopathological outcomes assessed. The correlation with follicular atresia was significant, with a coefficient of $r = 0.880$ ($p < 0.001$), indicating that a higher number of follicles is linked to greater follicular atresia. This trend was similarly observed with capsule thickening, which showed a correlation of $r = 0.880$ ($p < 0.001$). Theca cell hyperplasia also had a strong correlation with follicle count ($r = 0.840$, $p < 0.001$), suggesting that as the follicle count increases, there is a corresponding increase in the presence of hyperplasia in theca cells.

In contrast, stromal thickness showed no significant correlations with any of the histopathological features. The correlation coefficient with follicular atresia was $r = 0.190$ ($p = 0.239$), indicating no significant association. Similarly, stromal thickness did not correlate significantly with capsule thickening or theca cell hyperplasia, suggesting that while it is a measurable aspect of ovarian morphology, it may not directly reflect the underlying pathological changes associated with PCOS.

DISCUSSION

The area of research developed was intended to assess the ultrasonographic parameters and histopathological alterations in PCOS, more especially, this research established the strong link between ovarian volume, follicle count and histopathological findings including follicular atresia and capsule thickening. However, ovarian volume was significantly positively correlated with follicular atresia, $r = 0.891$, $p < 0.001$ and capsule thickening, $r = 0.891$, $p < 0.001$, this means that larger ovarian volume have more follicular atresia and capsule thickening. Another significant relatedness with follicle count was also observed (coefficient $r = 0.880$) which confirmed that as the number of the follicles rises, the rates of follicle atresia and capsule thickness also rise. This lack of correlation, however, was found with stromal thickness, which indicates that the latter may not be as helpful an ultrasonographic marker as the former three.

In the study by K Takahashi et al., they studied 20 patients and pointed out that ultrasound appearances do correlate well with histological findings, more so, this research has a larger sample size to give a more extensive review of ultrasound and histopathology of PCOS. The focus on the numerical relationships especially with respect to the analytical results of various PME organization patterns enrich the available knowledge base. In Takahashi's study, the qualification of ovarian morphology into General Cystic Pattern (GCP) and Peripheral Cystic Pattern (PCP) emphasized the usefulness of findings in ultrasound in determining the pathological aspect of PCOS.(29) Similarly, Ruo-Yan Zhu et al., pointed out that the characteristic features of USG in PCOS are enlarged ovaries, multiple small follicles, and increased stromal echogenicity. This supports the continued use of ultrasound in diagnosis but also recognises that polycystic ovaries are common in normal, asymptomatic women, thus clouding what is considered as diagnostic. These features make it even more important to discuss the differential diagnosis of ultrasound findings mentioned above, as similar features could be seen in other pathological conditions.(30)

In general, this research benefits the literature as it supports earlier research personnel in evaluating the implication of ovarian volume and follicle count in the diagnosis and management of PCOS. Further, the study also focuses on the issue of over interpretation of ultrasound results given the fact that majority of the population might present with sonographic features. In this synthesis of findings, the focus is placed on increasing the efficiency of diagnostic imaging methods when used in conjunction with histopathological methods, which may improve the quality of care for patients with PCOS. Therefore, other related studies should be conducted to analyze correlation between ultrasonographic changes and symptoms of PCOS, in order to gain better insight into the condition.

7.1: CONCLUSION:

In conclusion, this research highlights the vital role of ultrasonographic findings in diagnosing and understanding Polycystic Ovary Syndrome (PCOS). Significant correlations were identified between ovarian volume and follicle count with histopathological features such as follicular atresia and capsule thickening, indicating that these ultrasonographic parameters can serve as valuable indicators of ovarian health and dysfunction in affected patients. Larger ovarian volumes and higher follicle counts were associated with increased instances of follicular atresia, suggesting their relevance in assessing the morphological and functional aspects of the ovaries in PCOS. Furthermore, the study emphasizes the necessity for careful interpretation of ultrasound findings due to the prevalence of polycystic ovarian morphology in healthy women, aligning with existing literature that underscores the complexities involved in diagnosing PCOS. Overall, this research contributes to the broader understanding of the condition by confirming the importance of combining imaging techniques with histopathological analysis to enhance diagnostic accuracy and improve patient care for those affected by PCOS.

7.2: RECOMMENDATIONS:

It is crucial for clinicians to adopt a comprehensive approach to diagnosing Polycystic Ovary Syndrome (PCOS) by integrating ultrasonographic findings with histopathological evaluations. Utilizing a combination of parameters, such as ovarian volume and follicle count, alongside histopathological changes, can significantly improve diagnostic accuracy and reduce the likelihood of misdiagnosis.

7.3: LIMITATION:

One of the primary limitations of this study is the relatively small sample size of 40 patients. While the findings provide valuable insights into the correlation between ultrasonographic and histopathological features in PCOS, a larger sample size would enhance the generalizability of the results. Future studies with a more extensive patient cohort could provide a more comprehensive understanding of the relationships observed

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