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Article

Beyond Hormones: Metabolic and Environmental Drivers (Kallistatin, Zearalenone) in Menstrual Regularity of Polycystic Ovary Syndrome

Shams Al Duha Mazin Sattar[1], Rana Majeed Hameed[2*], Wasan Ghazi AlSafi[3]

1. Postgraduate student, Department of Biochemistry, College of Medicine, University of kerbala, Baghdad, Iraq
2. PhD Biochemist, Department of Biochemistry, College of Medicine, University of Kerbala, Kerbala, Iraq
3. Professor gynecologist, FICMS, CABOG, University of Kerbala, College of Medicine, Department of Obstetrics & Gynecology

* Correspondence: shamsalduha.m@s.uokerbala.edu.iq[1], rana.m@uokerbala.edu.iq[2], [3]Wasan.gh@uokeebala.edu.iq.com[3]

Abstract: Polycystic Ovary Syndrome (PCOS) is a heterogeneous endocrine disorder with diverse clinical manifestations, including menstrual irregularity. While its hormonal and metabolic aspects are well-studied, the combined effects of endogenous protective proteins like Kallistatin and environmental endocrine disruptors such as Zearalenone (ZEN) on menstrual regularity remain poorly understood. A cross-sectional study was conducted on 98 women diagnosed with PCOS using the Rotterdam criteria. Participants were classified into regular and irregular menstrual cycle groups based on clinical history. Serum levels of reproductive hormones, metabolic indices, Kallistatin, and ZEN were measured and compared between the groups. A p-value <0.05 was considered statistically significant. Women with irregular menstrual cycles exhibited more severe hormonal and metabolic disturbances. They had higher median testosterone (61 vs. 45 ng/dL) and lower SHBG levels (29 vs. 43 nmol/L). Metabolic indices also showed marked differences, with higher Visceral Adiposity Index (VAI: 4.2 vs. 2.4) and Lipid Accumulation Product (LAP: 119 vs. 94) in the irregular group. Notably, this group demonstrated significantly lower median Kallistatin levels (19 vs. 111 ng/mL) and higher ZEN levels (6.0 vs. 4.9 ng/L) compared to the regular-cycle group. The findings support the hypothesis that lower Kallistatin and elevated ZEN levels contribute to reproductive and metabolic dysfunction in PCOS.

Keywords: Polycystic Ovary Syndrome, Menstrual irregularity, Kallistatin, Zearalenone (ZEN), metabolic indices.

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1. Introduction

Polycystic Ovary Syndrome (PCOS) is one of the most common and complex endocrine disorders affecting women of reproductive age. Characterized by a constellation of reproductive, metabolic, and hormonal abnormalities, PCOS affects approximately 8–13% of women globally, with significant regional and ethnic variations in its prevalence [1]. The syndrome presents with diverse clinical features, including menstrual irregularity, hyperandrogenism, anovulation, and polycystic ovarian morphology, often accompanied by insulin resistance, central obesity, and dyslipidemia.

Menstrual irregularity is one of the earliest and most prominent manifestations of PCOS. It reflects underlying disruptions in the hypothalamic–pituitary–ovarian (HPO) axis and is tightly linked to anovulation and infertility. In PCOS, increased GnRH pulse frequency leads to preferential LH secretion, resulting in an elevated LH:FSH ratio and

excessive ovarian androgen production. This hormonal imbalance impairs normal folliculogenesis, ultimately manifesting as oligomenorrhea or amenorrhea. Moreover, the degree of menstrual disturbance often correlates with the severity of hyperandrogenism and metabolic dysfunction, making it a valuable clinical indicator of disease phenotype and progression [2].

Beyond intrinsic hormonal imbalances, growing evidence suggests that environmental endocrine disruptors may contribute to the etiology and severity of PCOS. One such compound is Zearalenone (ZEN), a mycoestrogen produced by Fusarium species that commonly contaminates food supplies. Due to its structural similarity to 17β-estradiol, ZEN can bind to estrogen receptors and interfere with the reproductive axis, potentially exacerbating menstrual irregularity and infertility in susceptible women [3-4].

At the same time, endogenous protective factors such as Kallistatin, a serine protease inhibitor with anti-inflammatory and anti-angiogenic properties, may play a counter-regulatory role in PCOS. Kallistatin has been shown to modulate oxidative stress, vascular remodeling, and metabolic homeostasis, all of which are relevant to ovarian function. However, limited research exists on its clinical relevance in PCOS, particularly in relation to reproductive outcomes and infertility subtypes.

Despite the growing bulk of literature on the hormonal and metabolic profiles of PCOS, the combined roles of environmental toxins like ZEN and endogenous regulators like kallistatin remain underexplored. Specifically, it is unclear how these characteristics relate to normal menstrual cycles, which are a sign of reproductive health.

Recent studies have emphasized the importance of menstrual cycle characteristics in understanding the underlying heterogeneity of PCOS. Irregular menstruation in PCOS reflects not only anovulatory dysfunction but also the degree of hyperandrogenism and metabolic disturbance. For instance, women with irregular cycles tend to exhibit elevated serum testosterone, reduced SHBG, and increased LH:FSH ratios—all indicative of impaired follicular maturation and hormonal imbalance [5]. These patterns vary across PCOS phenotypes, with classic hyperandrogenic forms (Phenotype A and B) showing more severe endocrine disruption compared to non-hyperandrogenic forms (Phenotype D) [1].

Beyond intrinsic hormonal dysregulation, the role of environmental toxins in exacerbating PCOS features has gained increasing attention. Zearalenone (ZEN), a non-steroidal estrogenic mycotoxin, has been implicated in reproductive toxicity through its ability to mimic estradiol and bind to estrogen receptors [3]. Experimental studies have shown that ZEN disrupts ovarian steroidogenesis, reduces oocyte quality, and alters the hypothalamic–pituitary–gonadal axis. Moreover, chronic dietary exposure to ZEN has been linked to menstrual irregularity, infertility, and hormonal imbalance in animal models and, to a lesser extent, in human observational studies [4-6].

In parallel, kallistatin—a hepatokine encoded by the SERPINA4 gene—has emerged as a potential biomarker in metabolic and inflammatory disorders. Its anti-inflammatory, anti-angiogenic, and antioxidant actions may contribute to ovarian health by modulating local vascularization and reducing oxidative stress. Studies suggest that reduced kallistatin levels may be associated with insulin resistance, endothelial dysfunction, and reproductive impairment [7-8]. However, research on kallistatin in the context of PCOS remains scarce, and its relationship with menstrual regularity and infertility has yet to be systematically examined.

Few studies to date have explored the interplay between environmental disruptors and endogenous protective proteins in PCOS. Investigating both ZEN and kallistatin within the same clinical population offers a unique opportunity to elucidate multifactorial influences on reproductive function, particularly in relation to cycle regularity and infertility subtypes.

Collectively, existing literature underscores the need for integrated approaches that consider hormonal, metabolic, environmental, and molecular factors to better classify PCOS phenotypes and identify new diagnostic targets. This study builds on these gaps by examining serum levels of ZEN and kallistatin in relation to menstrual cycle patterns and infertility classifications among Iraqi women with PCOS.

Zearalenone and kallistatin levels in women with PCOS will be evaluated, and their relationship to normal menstruation will be investigated. This study aims to give a more thorough understanding of the complex role behind menstruation dysfunction in PCOS by combining hormonal, metabolic, and environmental factors.

2. Materials and Methods

Patients: This cross-sectional study was conducted at the Department of Biochemistry, Kerbala Medical College, in collaboration with the outpatient clinic and the Obstetrics and Gynecology Hospital in Karbala, as well as the Baghdad Teaching Hospital (Medical City). The study protocol received ethical approval from the Scientific Committee of the College of Medicine, University of Karbala. A total of 98 women aged between 18 and 44 years, diagnosed with Polycystic Ovary Syndrome (PCOS), were enrolled. Participants were recruited based on clinical and laboratory assessments, and written informed consent was obtained prior to sample collection. Throughout the study, strict confidentiality and data privacy were maintained to ensure participant protection.

Inclusion Criteria: Women diagnosed with PCOS according to the 2012 revised Rotterdam criteria were included. Diagnosis required the presence of at least two of the following features: clinical and/or biochemical signs of hyperandrogenism, oligo- or anovulation, and polycystic ovarian morphology as detected by ultrasound. Participants were further classified according to phenotypic presentations and infertility subtypes. Additionally, classification based on menstrual cycle regularity was employed, where regular menstrual cycles were defined as intervals ranging from 21 to 35 days consistently over the past six months. Irregular cycles encompassed oligomenorrhea (menstrual intervals greater than 35 days), amenorrhea (absence of menstruation for three months or longer), or variable cycle lengths. This classification facilitated analysis of the association between menstrual regularity and hormonal as well as metabolic parameters in women with PCOS.

Exclusion Criteria: Participants were excluded if they were pregnancy or breastfeeding status, had thyroid dysfunction, hyperprolactinemia, or congenital adrenal hyperplasia. Also, women with chronic disease such as diabetes mellitus, cardiovascular disease, autoimmune disorders, hepatic or renal dysfunction were excluded, as well as, history of corticosteroid use or hormonal medications in the past three months

Sample Collection and Processing: Fasting venous blood samples were collected during the early follicular phase of the menstrual cycle. Serum was separated by centrifugation and stored at –20°C until analysis.

Hormonal and Biochemical Assays: Reproductive hormones (FSH, LH, estradiol, total testosterone, prolactin) were measured using electrochemiluminescence immunoassay (ECLIA) systems (Cobas e 411, Roche Diagnostics, Germany). Free testosterone was assessed using a chemiluminescence analyzer (MAGLUMI 600, Snibe Diagnostics, China). Serum lipid profiles and fasting glucose were analyzed via an automated chemistry analyzer (SMART 120, GenoTek, USA).

Kallistatin Measurement: Serum kallistatin concentrations were quantified using the Elabscience® Human SERPINA4 (Kallistatin) ELISA Kit, following the manufacturer's instructions. Optical density was measured at 450 nm using a microplate reader. The assay sensitivity and range were consistent with the kit's specifications.

Zearalenone Detection: ZEN was first identified qualitatively via Thin Layer Chromatography (TLC), followed by quantitative determination using High Performance Liquid Chromatography (HPLC). This two-step approach ensured accurate detection and quantification of serum ZEN levels.

Anthropometric and Metabolic Indices: Anthropometric measurements included body mass index (BMI), waist-to-hip ratio (WHR), body adiposity index (BAI), and lipid accumulation product (LAP). Basal metabolic rate (BMR) and visceral adiposity index (VAI) were also calculated using standard clinical equations.

Ethical Approval: The study was approved by the Ethical Committee of the College of Medicine, University of Karbala. The ethical approval letter NO: 24-59 Date :2025/7/10

Statistical Analysis: Statistical analyses were performed using Pad Prism 9. The distribution of the data was checked using Shapiro-Wilk test as numerical means of assessing normality. Descriptive statistics were expressed as mean ± standard deviation (SD) for normally distributed variables or median (minimum–maximum) for skewed data. Comparisons between groups were performed using ANOVA or Kruskal–Wallis tests, followed by post hoc analysis where appropriate. A p-value less than 0.05 was considered statistically significant.

3. Results and Discussion

Result

Table 1 shown the prevalence of various clinical manifestations and their association with the four identified PCOS phenotypes: Classic PCOS (A, HA, PCO), Classic PCOS (A, HA), Ovulatory PCOS (HA, PCO), and Normo-androgenic PCOS (A, PCO).

Hirsutism was most prevalent in the Classic PCOS (A, HA, PCO) group, affecting 85% of individuals, followed by Classic PCOS (A, HA) at 64%. In contrast, Ovulatory PCOS (HA, PCO) and Normo-androgenic PCOS (A, PCO) showed lower rates of hirsutism at 47% and 53%, respectively. Acne was reported in 53% of the Classic PCOS (A, HA, PCO) group, 36% of the Classic PCOS (A, HA) group, 40% of the Ovulatory PCOS (HA, PCO) group, and 37% of the Normo-androgenic PCOS (A, PCO) group. Alopecia was most common in the Ovulatory PCOS (HA, PCO) group (53%) and Classic PCOS (A, HA) group (54%), but less so in the Normo-androgenic PCOS (A, PCO) group (32%).

Menstrual cycle regularity showed significant differences across phenotypes. An overwhelming majority of individuals in Classic PCOS (A, HA, PCO) (94%), Classic PCOS (A, HA) (100%), and Normo-androgenic PCOS (A, PCO) (95%) reported irregular menstrual cycles. In stark contrast, 73% of women with Ovulatory PCOS (HA, PCO) reported regular menstrual cycles, defining them as typically within 21-35 days over a 12-month period, while irregular cycles consistently fell outside this range.

A comparison between PCOS patients with regular and irregular menstrual cycles revealed significant differences in metabolic and molecular profiles. These differences reflect deeper endocrine and metabolic disturbances linked to menstrual dysfunction in PCOS.

Table 1. Clinical variables and association with phenotypes of PCOS

		Classic PCOS (A, HA, PCO)	Classic PCOS (A, HA)	Ovulatory PCOS (HA, PCO)	Normo-androgenic PCOS (A, PCO)
Hirsutism	***Yes***	*85%*	*64%*	*47%*	*53%*
	No	*15%*	*36%*	*53%*	*47%*
Acne	***Yes***	*53%*	*36%*	*40%*	*37%*
	No	*47%*	*64%*	*60%*	*63%*
Alopecia	***Yes***	*40%*	*54%*	*53%*	*32%*
	No	*60%*	*46%*	*47%*	*68%*
Abortion	***Yes***	*15%*	*27%*	*20%*	*16%*
	No	*85%*	*73%*	*80%*	*84%*
Regularity of mc.	***regular***	*6%*	*0%*	*73%*	*5%*
	irregular	*94%*	*100%*	*27%*	*95%*

Note: * Hirsutism, androgenetic alopecia, acne (dichotomously as presence or absence);

*Regular mc. typically within 2-20 days over 12 months. Irregular, if the length consistently falls outside the 21-35 day range,

Table 2 & Figure 1 illustrated the median (and range) of reproductive hormone levels in PCOS patients, categorized by whether they experience irregular or regular menstrual cycles (mc).

For Luteinizing Hormone (LH), patients with irregular menstrual cycles had a median of 8.3 mIU/ml (range: 1.3-28), while those with regular cycles showed a slightly lower median of 7.8 mIU/ml (range: 3.4-20). In contrast, Follicle-Stimulating Hormone (FSH) levels were notably higher in patients with regular menstrual cycles, with a median of 7.7 mIU/ml (range: 3.0-19), compared to 5.8 mIU/ml (range: 1.2-9.9) in those with irregular cycles.

Progesterone levels were relatively similar between the groups, with a median of 17 ng/ml (range: 5.2-93) for irregular cycles and 14 ng/ml (range: 7.5-32) for regular cycles. Testosterone levels, a key indicator of hyperandrogenism in PCOS, were distinctly higher in the irregular mc. group, with a median of 61 ng/dl (range: 5.0-143), as opposed to 45 ng/dl (range: 9.0-97) in the regular mc. group.

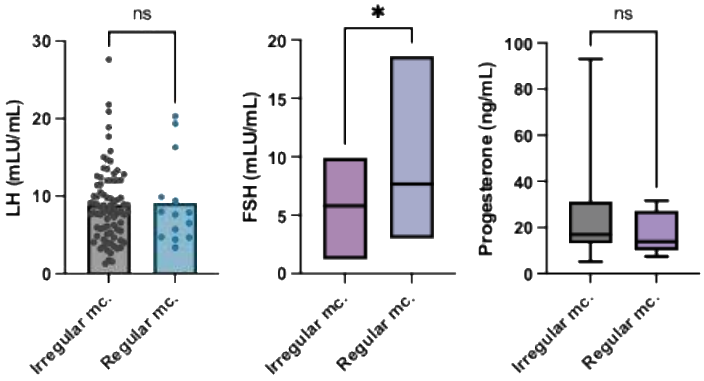
Sex Hormone Binding Globulin (SHBG) showed an inverse pattern to testosterone; patients with regular menstrual cycles had a higher median SHBG of 43 nmol/L (range: 34-47), whereas those with irregular cycles had a lower median of 29 nmol/L (range: 13-47). Finally, Estradiol (E2) levels were higher in the regular mc. group (median 68 pg/ml, range: 33-166) compared to the irregular mc. group (median 45 pg/ml, range: 29-173).

There was a clear difference in hormonal profiles based on menstrual cycle regularity in PCOS patients. Individuals with irregular menstrual cycles, a hallmark of PCOS, typically present with higher median Testosterone levels and lower SHBG levels compared to those with regular cycles. This hormonal imbalance (elevated androgens and reduced SHBG, which binds free androgens) is consistent with the anovulatory or oligo-ovulatory nature of irregular cycles in PCOS.

Conversely, patients with regular menstrual cycles, despite having PCOS, exhibit higher median FSH and E2 levels, and lower Testosterone and higher SHBG. The higher FSH in the regular cycle group likely indicates better follicular development and function. These distinctions underscore the heterogeneity of PCOS phenotypes and suggest that menstrual regularity is strongly associated with different underlying hormonal characteristics.

Table 2. Reproductive variables (Median (mini-max)) among PCOS patients groups based on regularity of mc.

	Irregular mc.	**Regular mc.**
LH (mIU/ml)	8.3 (1.3-28	7.8 (3.4-20
FSH (mIU/ml)	5.8 (1.2-9.9	7.7 (3.0-19
Progestrone. (ng/ml)	17 (5.2-93	14 (7.5-32
Testosterone (ng/dl)	61 (5.0-143	45 (9.0-97
SHBG (nmol/L)	29 (13-47	43 (34-47
E2 (pg/ml)	45 (29-173	68 (33-166



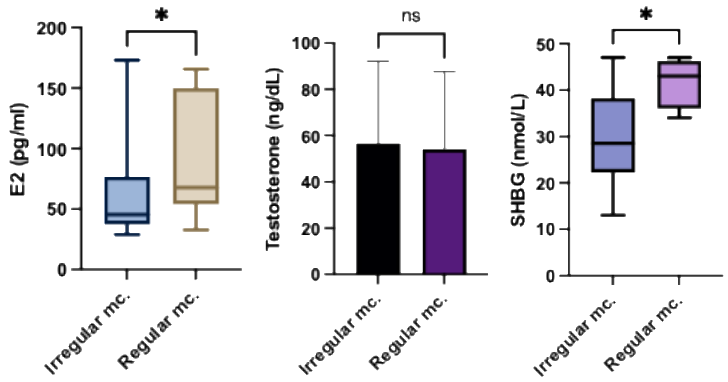


Figure 1. The distribution of serum Reproductive variables among PCOS patients groups based on regularity of mc. (t-test was *: significant at $p \leq 0.05$, **: significant at $p \leq 0.01$, ***: significant at $p \leq 0.001$, ****: significant at $p \leq 0.0001$)

Table 3 & Figure 2 presented the median (and range) values for metabolic indices, including body adiposity index (BAI), visceral adiposity index (VAI), lipid accumulation product (LAP), and basal metabolic rate (BMR), among PCOS patients categorized by irregular versus regular menstrual cycles (mc).

The Body Adiposity Index (BAI) showed identical median values for both groups, with 32 for patients with irregular mc. (range: 19-70) and 32 for those with regular mc. (range: 24-45), suggesting that overall body fat relative to height is comparable regardless of menstrual regularity.

In contrast, the Visceral Adiposity Index (VAI) differed significantly. Patients with irregular menstrual cycles had a substantially higher median VAI of 4.2 (range: 1.7-5.8), compared to 2.4 (range: 1.9-3.4) for those with regular cycles. This indicates a greater accumulation of visceral fat in individuals experiencing irregular menstruation.

Similarly, the Lipid Accumulation Product (LAP) was higher in the irregular mc. group, with a median of 119 (range: 30-583), compared to 94 (range: 28-189) in the regular mc. group.

Basal Metabolic Rate (BMR) showed relatively similar median values, with 1422 for the irregular mc. group (range: 1145-1904) and 1470 for the regular mc. group (range: 1217-1768). The ranges, however, indicate considerable individual variability within each category.

The findings from Table 3 highlight that while overall body adiposity (BAI) appears similar, there are notable metabolic differences between PCOS patients based on their menstrual regularity. Patients with irregular menstrual cycles demonstrate higher levels of both Visceral Adiposity Index (VAI) and Lipid Accumulation Product (LAP).

This indicated that irregular menstruation in PCOS is strongly associated with a less healthy metabolic profile, characterized by greater visceral fat accumulation and potentially higher lipid-related risk. The higher VAI and LAP in the irregular cycle group are consistent with the known metabolic disturbances often seen in the more severe phenotypes of PCOS.

The BMR, while slightly different in median, shows substantial overlap in ranges, showing less distinct variation compared to VAI and LAP. These results illustrated the importance of assessing metabolic health, especially visceral adiposity and lipid accumulation, in PCOS patients, particularly those presenting with menstrual irregularities.

Table 3. Biochemical evaluation of metabolic incised (Median (mini-max)) among PCOS groups based on regularity of mc.

	Irregular mc.	**Regular mc.**
BAI	32 (19-70	32 (24-45
VAI	4.2 (1.7-5.8	2.4 (1.9-3.4

LAP	*119 (30-583*	*94 (28-189*
BMR	*1422 (1145-1904*	*1470 (1217-1768*

*body adiposity index (BAI), visceral adiposity index (VAI), lipid accumulation product (LAP), and basal metabolic rate (BMR)

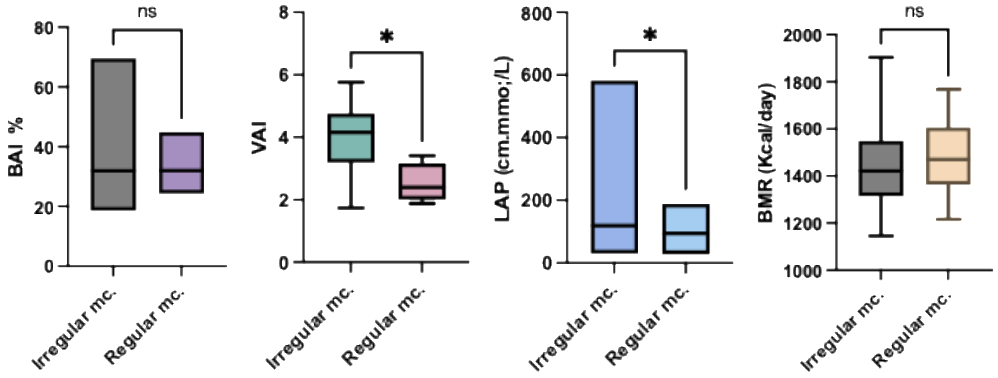


Figure 2. The distribution of evaluation metabolic incised among PCOS patients groups based on regularity of mc. (t- test was *: significant at $p \leq 0.05$, **: significant at $p \leq 0.01$, ***: significant at $p \leq 0.001$, ****: significant at $p \leq 0.0001$)

Table 4 & Figure 3 demonstrated the median (and range) levels of Kallistatin and Zearalenone in PCOS patients, categorized by menstrual cycle (mc) regularity.

Kallistatin levels showed considerable variation across the groups. The Healthy Control group exhibited the highest median Kallistatin level at 117 (range: 16-342). Among PCOS patients, those with regular menstrual cycles had a notably higher median Kallistatin level of 111 (range: 31-121), which is very close to that of healthy controls. In stark contrast, PCOS patients with irregular menstrual cycles displayed a significantly lower median Kallistatin level of 19 (range: 2-271).

For Zearalenone, the median levels were highest in PCOS patients with irregular menstrual cycles, at 6.0 ng/L (range: 2.3-16). Patients with regular menstrual cycles had a lower median of 4.9 ng/L (range: 2.1-10), while the Healthy Control group showed the lowest median Zearalenone level at 4.2 ng/L (range: 2.1-8.2).

PCOS patients with regular menstrual cycles exhibit Kallistatin levels remarkably similar to those of healthy controls, suggesting that favorable Kallistatin levels might be associated with preserved ovarian function and regular ovulation despite a PCOS diagnosis. Conversely, the significantly lower Kallistatin levels observed in PCOS patients with irregular menstrual cycles point to a potential role for Kallistatin as a biomarker for more severe ovulatory dysfunction within PCOS. This could imply that lower Kallistatin contributes to or reflects the underlying physiological mechanisms leading to menstrual irregularity in PCOS.

The elevated median Zearalenone levels in PCOS patients with irregular menstrual cycles compared to both regular mc. PCOS patients and healthy controls is noteworthy. Zearalenone, being a mycoestrogen, can exert estrogenic effects. Its higher presence in irregular cycle patients might reflect an environmental factor contributing to endocrine disruption and exacerbating menstrual irregularities in PCOS.

Table 4. Mean level of Kallistatin and Zearalenone (Median (mini-max)) among PCOS patients groups based on regularity of mc.

	Irregular mc.	**Regular mc.**	**Healthy Control**
Kallistatin	19 (2-271)	111 (31-121)	117 (16-342)
Zearalenone ng/L	6.0 (2.3-16)	4.9 (2.1-10)	4.2 (2.1-8.2)

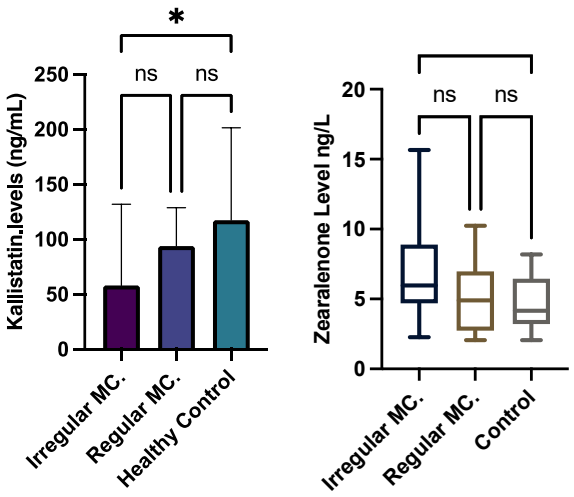


Figure 3. The distribution of serum level of Kallistatin and Zearalenone among PCOS patients groups based on regularity of mc. (Post Hoc ANOVA test was *: significant at $p \leq 0.05$, **: significant at $p \leq 0.01$, ***: significant at $p \leq 0.001$, ****: significant at $p \leq 0.0001$)

Discussion

The analysis in Table 2 examines the variation of hormonal, metabolic, and biochemical markers in connection to menstrual cycle regularity, based on findings associated with infertile status. This study provides further insights into the hormonal and metabolic variability of PCOS, especially concerning ovulatory function.

Table 2 presents a comparative overview of reproductive hormones in PCOS patients, categorized according to menstrual cycle regularity. The data show that patients with irregular cycles tend to have higher mean testosterone levels (61 ng/dL) and lower SHBG levels (29 nmol/L), compared to those with regular cycles (testosterone: 45 ng/dL; SHBG: 43 nmol/L). This hormonal disparity strongly suggests hyperandrogenism and increased androgen bioavailability, characteristics of PCOS in its most severe forms and anovulation.

The observed inverse relationship between testosterone and SHBG is consistent with the findings of Li et al. [9], who confirmed that low SHBG promotes high levels of circulating free testosterone, thus exacerbating clinical symptoms such as hirsutism and menstrual irregularity.

Furthermore, FSH and estradiol (E2) levels were significantly higher in women with regular menstrual cycles (FSH: 7.7 mIU/ml, E2: 68 pg/ml) compared to women with irregular menstrual cycles (FSH: 5.8 mIU/ml, E2: 45 pg/ml). This pattern supports the idea that regular menstrual cycles in PCOS patients may indicate persistent or impaired ovulatory activity, as also discussed in Rashid et al. [10]. In this context, increased FSH levels may enhance follicular growth and estrogen production, potentially stabilizing menstrual cycles despite underlying PCOS. In contrast, LH levels were elevated in both groups, with the group exhibiting irregular menstrual cycles showing slightly higher levels (8.3 vs. 7.8 mIU/ml), indicating persistent neuroendocrine dysregulation. Prolactin levels exhibited minor variations, yet remained elevated in both groups, indicating a potential subtle role in ovulatory dysfunction among these patients.

Table 3 complements the hormonal findings related to menstrual regularity by focusing on metabolic indices. This transition allows for a deeper exploration of how cycle regularity may reflect or interact with underlying metabolic risk in women with PCOS.

As shown in Table 3, patients with PCOS and irregular menstrual cycles showed significantly higher metabolic markers, particularly the visceral adiposity index (VAI) and lipid accumulation index (LCI). The mean VAI in the irregular cycle group was 4.2, while

the LCI was 119, both significantly higher than the VAI in the regular cycle group (VAI: 2.4; LCI: 94). These findings suggest that menstrual irregularity could be a clinical indicator of a deeper metabolic disorder.

These findings are consistent with those of [11], who identified a strong association between increased visceral adiposity, insulin resistance, and ovulatory dysfunction in PCOS. Higher AVF and PAF could reflect increased central obesity, which is known to exacerbate androgen excess and impair folliculogenesis, thus contributing to irregular cycles.

Interestingly, body adiposity index (BAI) values were similar between both groups (median: 32), suggesting that total body fat alone may not explain the variation in metabolic risk. Rather, fat distribution, particularly visceral fat, appears to play a larger role, consistent with the findings of [12].

Basal metabolic rate (BMR) showed only minor differences, with slightly higher medians in the regularly cycling group (1470 kcal/day) compared to the irregularly cycling group (1422 kcal/day). Although this finding is not statistically significant, it could indicate differences in body composition or energy metabolism that influence, or are influenced by, hormonal fluctuations and menstrual cycle regularity.

Taken together, these data suggest that menstrual regularity in women with PCOS is not simply a reproductive indicator but also reflects the underlying metabolic status. Patients with irregular cycles are more likely to have inappropriate adiposity and fat accumulation, reinforcing the multifaceted nature of PCOS.

Building on the observed metabolic disparities, Table 4 examines how menstrual regularity correlates with key molecular markers—Kallistatin and Zearalenone—to further elucidate their role in PCOS pathogenesis.

Table 4 shows the distribution of kalistatin and zearalenone (ZEN) levels according to menstrual cycle regularity in PCOS patients. Women with irregular menstrual cycles showed significantly lower kalistatin levels (median: 19 ng/ml) and higher ZEN concentrations (median: 6.0 ng/L) compared to women with regular menstrual cycles (kalistatin: 111 ng/ml; ZEN: 4.9 ng/L). The control group consistently showed the best values (kalistatin: 117 ng/ml; ZEN: 4.2 ng/L).

The findings represent the dual influence of inflammatory and environmental factors on reproductive dysfunction in patients with PCOS. Lower kalistatin levels in women with irregular menstrual cycles may indicate increased oxidative stress and subclinical inflammation, both of which are recognized mechanisms that can hinder follicular growth. Zhao et al [13] identified kalistatin as an anti-inflammatory molecule that is frequently decreased in women experiencing ovulatory dysfunction and metabolic syndrome.

Previous study link PCOS to lower Kallistatin, suggesting impaired vascular protection. Others report Kallistatin is elevated in PCOS, particularly in obese or highly insulin-resistant phenotypes, as a compensatory mechanism against inflammation. Based on these finding which strongly suggests that severe PCOS presentation (Irregular mc) is associated with a depletion or failure of Kallistatin's protective role, potentially linked to higher metabolic or inflammatory stress in this specific subgroup. That was confirmed by Yurtkal et al and others who have explored Kallistatin's link to cardiovascular and metabolic diseases, which are highly prevalent in PCOS. The conflict often arises from differences in patient BMI, degree of insulin resistance, and ethnic background [14].

The elevated ZEN levels in the group of women with irregular menstrual cycles were consistent with the findings of Rashid et al [15], who reported that chronic exposure to zearalenone, a mycoestrogen, may disrupt hypothalamic signaling and contribute to menstrual irregularity. This association between low kalistatin and high ZEN may suggest

a synergistic pathway, whereby environmental toxins and decreased protective proteins together exacerbate reproductive and metabolic dysfunction in PCOS.

Research increasingly links Zearalenone (a fungal toxin with powerful estrogenic activity) to reproductive disorders and hormone disruption. Studies on animal models and emerging human data suggest Zearalenone exposure can disrupt the hypothalamic-pituitary-ovarian (HPO) axis. These finding supports the hypothesis that environmental xenoestrogens, like Zearalenone, may contribute to the severity of PCOS phenotypes, specifically those characterized by chronic anovulation (irregular mc). Higher levels correlate with more severe endocrine disruption. That was consistence with Ly et al), who highlights Zearalenone's classification as an endocrine-disrupting chemical (EDC) that binds to estrogen receptors, potentially exacerbating the hormonal imbalance (hyperandrogenism and anovulation) central to PCOS [16].

4. Conclusion

This study highlights significant metabolic and differences between PCOS patients with regular and irregular menstrual cycles. While overall body adiposity (BAI) was similar, women with irregular cycles demonstrated higher levels of visceral adiposity (VAI) and lipid accumulation (LAP), indicating a more adverse metabolic profile. These findings support the well-established link between central obesity and reproductive dysfunction in PCOS. Irregular menstruation was associated with lower kallistatin levels, suggesting a possible role for this anti-inflammatory protein in preserving hormonal balance and ovulatory function. In contrast, higher serum ZEN concentrations in women with irregular cycles point to the potential impact of environmental endocrine disruptors on reproductive health. Together, these results underscore the multifactorial nature of menstrual irregularity in PCOS, involving both endogenous regulatory deficits and exogenous toxic exposures. Kallistatin may serve as a promising biomarker for identifying women at higher risk of cycle disruption and infertility, while elevated ZEN levels highlight the need for awareness of dietary and environmental influences. Future research should further explore the mechanistic pathways linking kallistatin and ZEN to menstrual and reproductive outcomes, and assess their potential utility in diagnosis, phenotype stratification, and targeted interventions for women with PCOS.

Statement and Declaration
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