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RESEARCH ARTICLE
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EFFECT OF DIANE-35 AND METFORMIN ON LIVER AND KIDNEY OF POLYCYSTIC OVARIAN SYNDROME PATIENTS

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Abstract

Polycystic ovarian syndrome (PCOS) is one of the most common endocrinal disorders of women of the reproductive age. It is associated with overweight, menstrual irregularities, change in luteinizing, and follicle-stimulating hormone ratio. Diane 35 and Metformin are used for polycystic ovarian syndrome patients to control body mass index, oligomenorrhea, mood changes, acne, and hirsutism levels and increase the chances of conception. The study aims to determine the effects of Diane 35 and metformin on CBC, LFTS, RFTS, L, and LH in the blood of polycystic ovarian syndrome patients. This research was conducted at Sahiwal Medical College, Punjab Pakistan. All the selected patients were screened at The University of Lahore. It was a prospective study in which 50 PCOS women were included by taking their Informed consent. Non-probability convenience sampling technique was used. Group I was the control group, Group II was administered metformin, Group III was administered Diane-35 and Group IIV was treated with Diane-35 and metformin for three months. All groups' preliminary weight and biochemical tests had been measured. Statistically analyzed results showed that P-value <0.05 and mentioned the age of groups II, III and IV. The mean age of the participants in the group I was 24.00±3.80 years, group II was 25.53±6.41, group III was 29.73±2.37 years and group IV were 29.26±2.84 years. The mean BMI of the participants in the group I was 22.02±3.70, group II was 27.92±7.036 group III was 26.97±6.35 and group IV was 27.08±5.96.

In all groups there were 86.7% participants with a history of acne and 2 (13.2%) without a history of acne. There was no significant difference in the mean values of the follicular stimulating hormone and serum luteinizing hormone in different groups. In the group II, there was 1 participant with multiple small follicles in both ovaries, 1 6.7% with multiple cysts, and 60% with PCO. In group II, there was 13.3% participants with a cyst in the ovary and 13.3% with cystic ovaries 6.7% participant with multiple small follicles in both ovaries, 6.7% with multiple cysts, and 46.7% with PCO. In group III there were 26.7% participants with cystic ovaries, 60% with PCO. Our results showed that treating PCOS women with Diane-35 and metformin reduced pathological alterations. Ultimately, PCOS affects women of childbearing age and has negative consequences for fertility and conception by interfering with hormone levels and the timing of ovulation.

Keywords: Polycystic ovarian syndrome; Diane-35; Metformin; Luteinizing hormone, Follicle stimulating hormone.

Introduction

Polycystic ovary syndrome (PCOS) is a significant metabolic condition and endocrine disorder that affects 8-13% of reproductive-aged women worldwide, making it one of the primary causes of infertility. Clinically, PCOS manifests through hyperandrogenism (such as hirsutism and acne), menstrual irregularities, ovulatory dysfunction, reproductive endocrine hormone imbalances, insulin resistance, and dyslipidemia (Huan *et al*., 2021). Dysfunction in the hypothalamic-pituitary-ovarian axis is strongly linked to PCOS, leading to increased gonadotropin-releasing hormone pulse frequency and greater luteinizing hormone (LH) synthesis compared to follicle-stimulating hormone (FSH) secretion (Biswas, 2019). This imbalance often results in ovarian follicular atresia and hyperandrogenemia due to reduced FSH levels, which hampers follicular growth and aromatase activity (Liang *et al*., 2023). Additionally, elevated insulin levels in PCOS patients may enhance LH expression and stimulate ovarian and adrenal androgen production, contributing to the syndrome (Ma *et al*., 2021).

PCOS symptoms include hyperandrogenism, persistent oligo/anovulation, polycystic ovaries, obesity, and metabolic abnormalities such as insulin resistance with compensatory hyperinsulinemia, affecting 14-18% of reproductive-age women globally (Escobar-Morreale, 2018). There is also an association between PCOS and endometrial dysfunction, including endometrial hyperplasia and endometrial cancer, due to imbalances in estrogen and progesterone levels (Shen *et al*., 2021). Young women with PCOS have a fourfold increased risk of developing endometrial cancer compared to those without PCOS. This elevated risk is influenced by changes in endogenous hormone metabolism, obesity, and insulin resistance (Y. Zhang *et al*., 2020).

The management of PCOS typically involves reducing insulin resistance using medications such as metformin. This approach has become standard practice due to its effectiveness in improving insulin sensitivity and lowering hyperandrogenism (Manna *et al*., 2023). Historically, PCOS treatment included combinations of ethinylestradiol and cyproterone acetate to protect the endometrium, regulate the menstrual cycle, and reduce symptoms like hirsutism and acne caused by hyperandrogenism (Krishna, 2019). Recent research suggests that combining Diane-35, which contains these compounds, with Bailing capsules (derived from fermented Cordyceps sinensis) may enhance treatment efficacy. Bailing capsules offer benefits such as lowering cholesterol and glucose levels, and safeguarding liver and kidney function (Okamura *et al*., 2017). Some studies recommend using Bailing capsules as an adjuvant treatment for PCOS (Hussein & Soslow, 2018).

PCOS prevalence varies widely, affecting between 6% and 21% of women, depending on race, environment, and genetic factors. The clinical presentation of PCOS often includes hyperandrogenism, polycystic ovaries, and anovulation, with common complications such as obesity and insulin resistance (Braun *et al*., 2016). The importance of early detection and effective multidisciplinary therapy for PCOS is underscored by its association with increased risks of

metabolic, cardiovascular, and gynecological malignancies. Lifestyle modifications, including diet and exercise, are crucial for managing PCOS (Sadeghi *et al*., 2022).

Diane-35, an oral contraceptive pill containing 35 micrograms of estrogen, is commonly prescribed for PCOS patients. It includes cyproterone acetate, a progestin that acts as an anti-androgen by inhibiting testosterone activity in the skin and other tissues, helping to alleviate symptoms such as acne and excessive hair growth (Sanchez-Rangel & Inzucchi, 2017). Metformin, initially developed for diabetes treatment, has also been adopted for PCOS due to its ability to improve insulin sensitivity and lower androgen levels. PCOS treatment often involves a combination of medications, including hormonal contraceptives, metformin, myoinositol, orlistat, and GLP1 agonists (Liu *et al*., 2022). Diane-35, particularly, is favored in China for its combined formulation of ethinylestradiol and cyproterone acetate, which helps manage hyperandrogenism and its clinical manifestations like acne, seborrhea, and hirsutism (Rena *et al*., 2017). Metformin, on the other hand, targets metabolic symptoms, enhancing glucose uptake and utilization, and decreasing gluconeogenesis in the liver (Liao *et al*., 2024).

While Diane-35 is effective in controlling hyperandrogenism and menstrual irregularities, concerns about its safety, particularly regarding liver function, have been raised. Cyproterone acetate has been linked to potential liver lesions and carcinogenesis, necessitating caution in patients with compromised liver function (Abadi *et al*., 2023). The combination of Diane-35 and metformin has shown promising results in treating PCOS, improving clinical symptoms, and enhancing ovulation and menstrual regularity. However, more research is needed to fully understand the molecular mechanisms and long-term effects of this combined therapy (Adelakun *et al*., 2024). This study aims to explore the effects of combining metformin and Diane-35 on ovulation and energy metabolism in a rat model of polycystic ovary syndrome, potentially shedding light on new therapeutic approaches for PCOS.

Materials and Methods

The study was a prospective analysis conducted at Sahiwal Medical College, Pakistan, to evaluate the combined effects of Diane-35 and metformin on liver and kidney function in unmarried females with PCOS. A non-probability convenience sampling technique was used to recruit 50 female participants, including 45 unmarried females with PCOS and 5 healthy unmarried women as controls. Participants, aged 19-48 years, were diagnosed based on clinical and biochemical markers, and randomly assigned to four groups: control, metformin, Diane-35, and combined Diane-35 and metformin. Group I served as the control group consisting of healthy unmarried females; Group II received metformin; Group III was administered Diane-35; and Group IV was treated with both Diane-35 and metformin. The treatment period lasted for three months. Before the initiation of the treatments, baseline measurements were recorded for all participants. These included body weight, liver function tests (LFTs), renal function tests (RFTs), complete blood count (CBC), fasting blood sugar levels, and ultrasonographic images of the ovaries. The specific biochemical tests for LFTs included Alanine transaminase (ALT), Aspartate transaminase (AST), total and free bilirubin, albumin, and alkaline phosphatase. RFTs measured urea and creatinine levels, while CBC evaluated total leukocyte count (TLC), differential leukocyte count (DLC), hemoglobin levels, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet count. Ethical approval was obtained from the University of Lahore's review committee, and informed consent was secured from all participants. Blood samples were collected before and after the three-month treatment period, processed to separate serum, and analyzed for biochemical markers. Data on clinical symptoms, dietary habits, and biochemical results were collected using a structured questionnaire. Statistical analysis was performed using One-way ANOVA in SPSS, with a p-value of less than 0.05 considered statistically significant.

Results

In table 1, the mean age of the participants was 24.00±3.80 years in group I. The minimum age was 20 years, and the maximum was 28 years. The mean age of the participants in group II was 25.53±6.41 years. The minimum age was 14 years, and the maximum was 37 years. In group III, the mean age of the participants was 29.73±2.37 years. The minimum age was 21 years, and the maximum was 33 years. In group IV, the mean age of the participants was 29.26±2.84 years. The minimum age was 21 years, and the maximum was 33 years (Figure 1).

Table 1: Age & BMI of study participants in all groups

	Age	Height	Weight	Body Mass Index
Group I	24.00±3.80	5.44±0.16	60.40±8.98	22.02±3.70
Group II	25.53±6.41	5.24±.021	71.73±20.13	27.92±7.036
Group III	29.73±2.37	5.38±0.077	72.60±17.43	26.97±6.35
Group IV	29.26±2.84	5.38±0.077	73.00±16.70	27.08±5.96

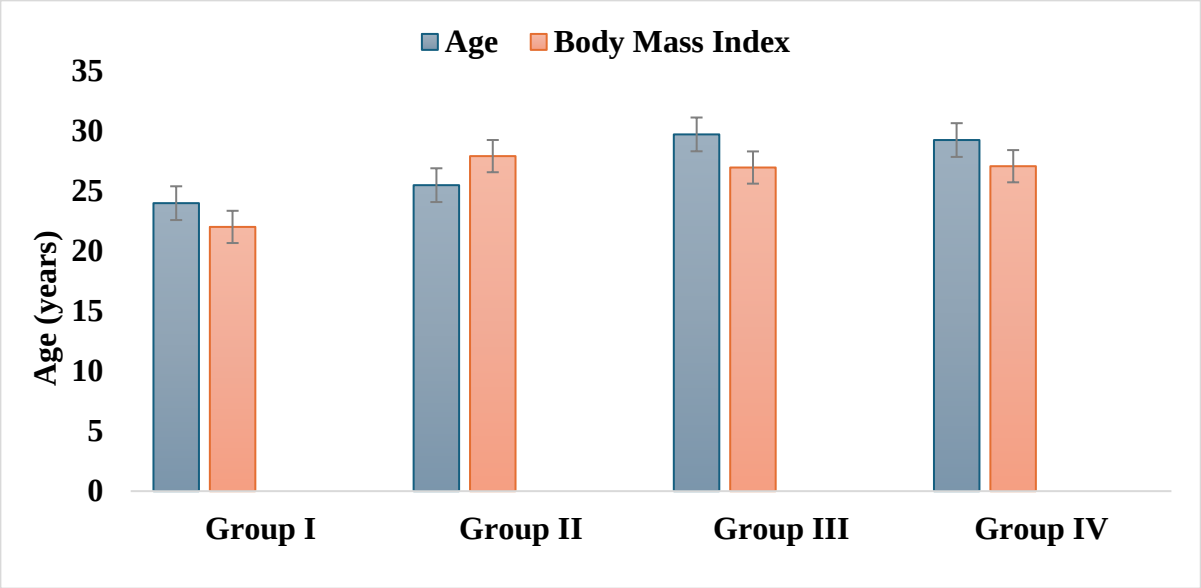

Figure 1: Age and BMI of study Participants in all groups

History of physiological parameters among study participants

In table 2 and 2.1, there were 100% participants with a history of weight gain and no participant without a history of weight gain. In different groups there were 86.7% participants with history of acne and 13.2% without history of acne while in normal healthy group there was no participant with history of acne. In different groups there were 93.3% participants with history of hirsutism and 6.7% without history of hirsutism while in normal healthy group there was no participant with history of hirsutism. In group I there was no participant with distribution of hairs. In group II there were 33.3% participants with distribution of hair, in group III and IV there were 33.3% participants with distribution of hair and 66.7% without distribution of hairs. In group I there were no participants with depressed mode. In groups II, III and IV there were 93.3% participants with depressed mode and 6.7% without depressed mode (Table 2.1). In group I there were no participants with sleep disturbance. In groups II, III and IV there were 60% participants with sleep disturbance and 6(40%) without sleep disturbance. In group I there was no participant with hyperpigmentation. In groups II, III and IV there were 73.3% participants with Hyperpigmentation and 26.7% without hyperpigmentation. In all the different groups there were 100% participants each with headache and in the normal healthy group no participant with headache. In group I there was no participant with abdominal bloating. In groups II, III and IV there were 80%, 73.3% and 80% participants with abdominal bloating. In group I there was no participant with history of surgery. In group II, III and IV there were 40% participants with history of surgery. In group I there was no participant with

history of diabetes. In groups II, III and IV there were 13.3%, 20% and 20% participants respectively with history of diabetes.

Table 2: History of physiological parameters among study participants

	Weight Gain %		Acne %		Hirsutism %		Distribution of hair %		History of Surgery %	
	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
Group I	100	0	0	100	0	100	0	100	0	100
Group II	100	0	86.7	13.2	93.3	6.7	33.3	66.7	40	60
Group III	100	0	86.7	13.2	93.3	6.7	33.3	66.7	40	60
Group IV	100	0	86.7	13.2	93.3	6.7	33.3	66.7	40	60

Table 2.1: History of physiological parameters among study participants

	Depressed Mode %		Sleep Disturbance %		Hyperpigmentation %		Head Ache %		Abdominal Bloating %		History of DM %	
	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
Group I	0	100	0	40	0	100	0	100	0	100	0	100
Group II	93.3	6.7	60	40	73.3%	26.7	100	0	80	20	13.3	86.7
Group III	93.3	6.7	60	40	73.3%	26.7	100	0	73.3	26.7	20	80
Group IV	93.3	6.7	60	40	73.3%	26.7	100	0	80	20	20	80

Findings of ultrasonography

In Table 3, there was 1 participant with multiple small follicles in both ovaries in group II, 1 6.7% with multiple cysts, 60% with PCO and 13.3% with a small cyst in the ovary and 13.3% with Uterus A/v, N/V. In group III, there was 13.3% participants with a cyst in the ovary and 13.3% with cystic ovaries 6.7% participant with multiple small follicles in both ovaries, 6.7% with multiple cysts, 46.7% with PCO and 13.3% with Uterus A/v, N/V. In group IV there were 26.7% participants with cystic ovaries, 60% with PCO, 6.7% with a small cyst in the ovary and 6.7% with Uterus A/v, N/V (Figure 2).

Table 3: Ultrasonography findings percentage among participants

	Group I (%)	Group II (%)	Group III (%)	Group IV (%)
Cyst in ovary	0	0	13.3	0
Cystic ovaries	0	0	13.3	26.7
Multiple small follicles in both ovaries	0	6.7	6.7	0
Multiple Cysts	0	6.7	6.7	0
PCO	0	60	46.7	60.0
Small cyst in ovary	0	13.3	0	6.7
Uterus A/v, N/V	0	13.3	13.3	6.7

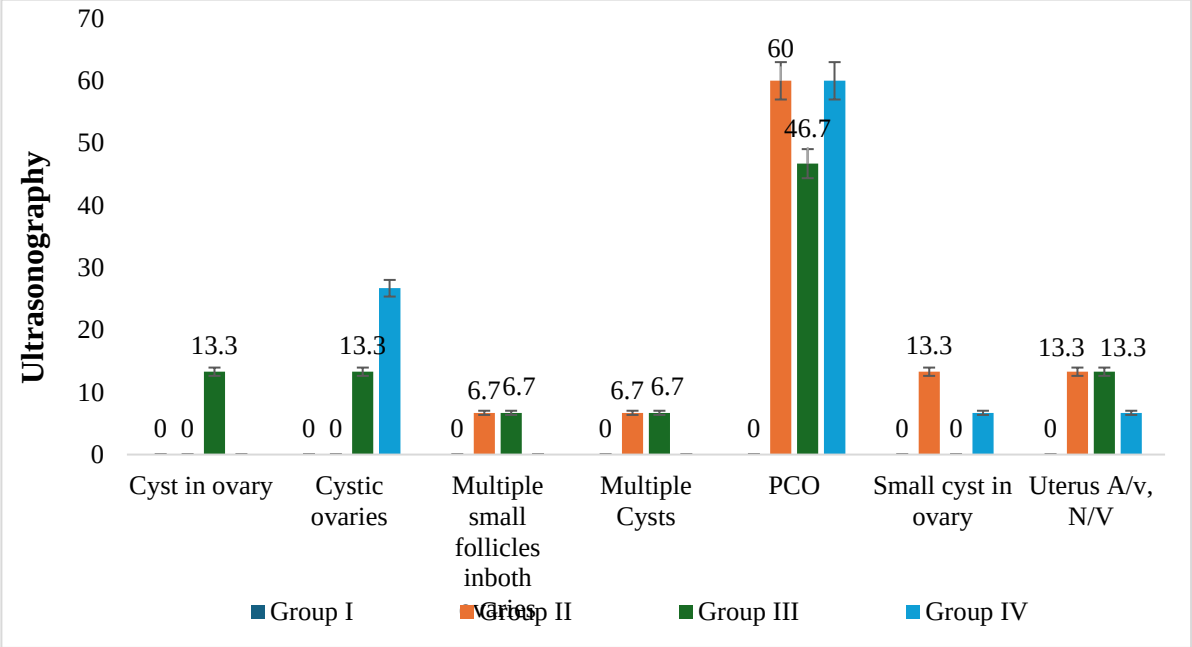

Figure 2: Ultrasonography findings percentage among participants

Level of biochemical markers in groups

In table 4, there was no significant difference in the mean values of FSH in treatment groups (*p-value*=0.339). There was no significant difference in the mean values of serum LH in treatment groups (*p-value*=0.063). There was a significant difference in the mean values of AST level in treatment groups (*p-value*<0.000). There was a significant difference in the mean values of ALT level in treatment groups (*p-value*<0.000). There was a significant difference in the mean values of Alkaline phosphatase level (ALP) in treatment groups (*p-value*<0.000) (Figure 3). There was a significant difference in the mean values of TLC in treatment groups (*p-value*=0.029). There was a significant difference in the mean values of hemoglobin levels in treatment groups (*p-value*<0.000). There was no significant difference in the mean values of PVC in treatment groups (*p-value*=0.167). There was no significant difference in the mean values of MCV in treatment groups (*p-value*=0.68). There was no significant difference in the mean values of MCH in treatment groups (*p-value*=0.69). There was a significant difference in the mean values of MCHC in treatment groups (*p-value*=0.99). There was a significant difference in the mean values of platelet count in treatment groups (*p-value*=0.007). There was significant difference in the mean values of urea in treatment groups (*p-value*:0.000). There was a significant difference in the mean values of creatinine levels in treatment groups (*p-value*=0.000). There was no significant difference in the mean values of Albumin level in treatment groups (*p-value*=0.000) (Figure 4).

Table 4: Different markers level after treatment in study groups

	Group I	Group II	Group III	Group IV	p-value
FSH	8.52±1.50	5.26±2.67	7.80±5.66	7.80±5.66	**0.339**
LH	14.00±4.35	6.97±5.10	10.76±5.80	10.76±5.801	**0.063**
ALT	20.40±1.14	28.61±2199	53.20±11.44	41.00±13.84	**<0.001**
AST	22.80±2.58	31.26±13.31	72.93±21.91	33.20±24.34	**<0.001**
ALP	61.20±7.56	244.86±37.93	409.10±83.63	326.60±81.60	**<0.001**
TLC	7.0±1.58	7.59±1.04	9.86±3.00	10.68±4.84	**0.029**
Hemoglobin	13±0.71	12.03±0.83	11.36±0.69	11.36±0.69	**<0.001**
PCV	37.80±1.92	55.78±10.67	52.18±17.26	50.24±18.88	**0.167**
MCV	80.00±3.00	74.00±19.46	80.22±15.60	80.22±15.60	**0.68**
MCH	28.80±2.16	27.78±2.25	27.06±3.73	27.06±3.73	**0.69**
MCHC	31.20±1.30	31.39±1.45	31.32±2.06	31.25±2.08	**0.99**
Platelet	310.60±58.58	286.26±74.60	247.33±22.35	234.4±37.79	**0.007**
Urea	26.0±4.18	21.66±8.32	50.73±9.09	49.93±11.96	**<0.001**
Creatinine	0.60±0.15	0.56±0.16	1.79±0.43	3.14±0.61	**<0.001**

Albumin	2.72±	5.38±	4.69±	3.99±	**0.43**

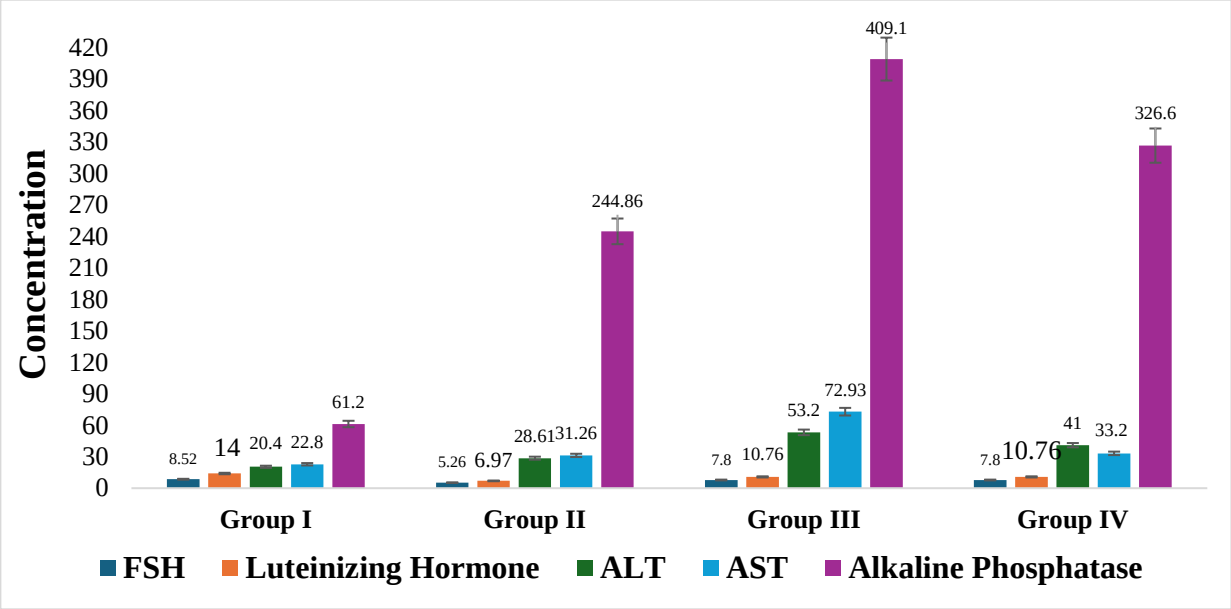

Figure 3: Level of biochemical markers in study groups

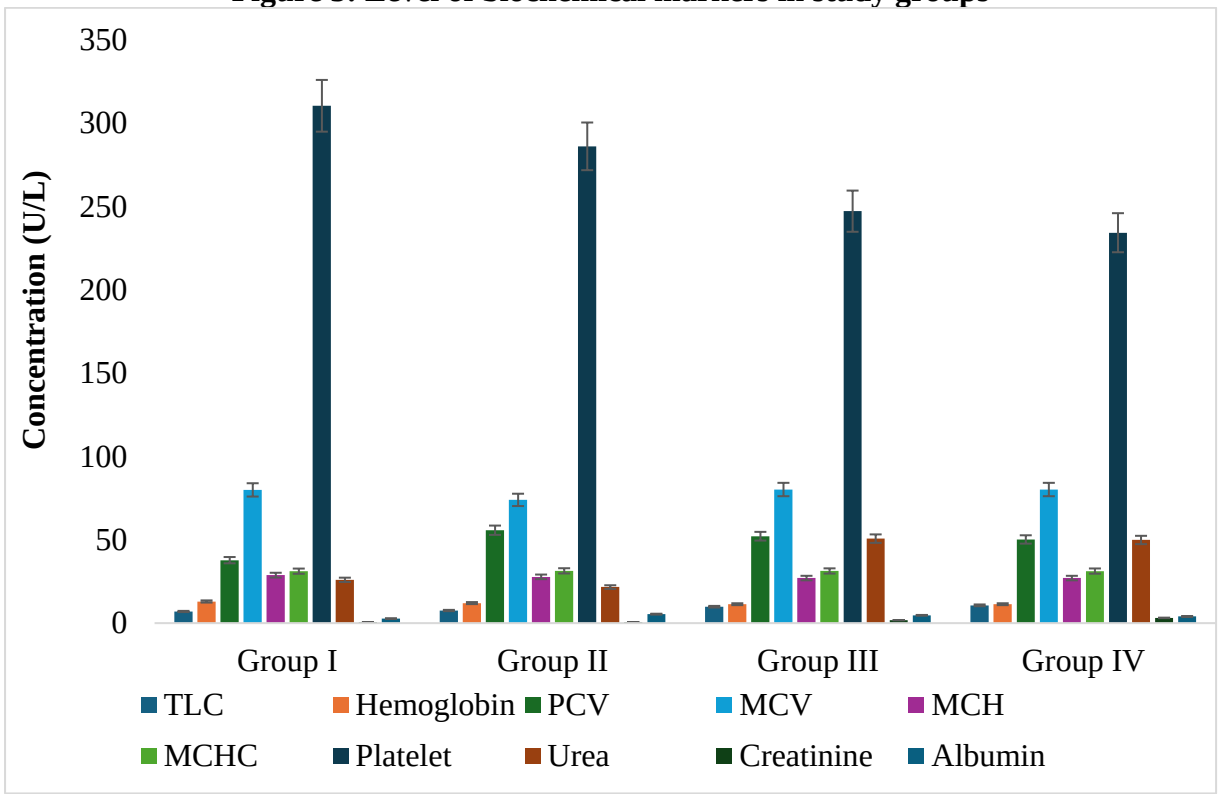

Figure 4: Level of biochemical markers in study groups

Discussion

PCOS is the leading cause of primary infertility in reproductive-aged women, affecting 8-13% of women worldwide. It is a complex endocrine and metabolic disorder characterized by hyperandrogenism (hirsutism, acne), menstrual irregularity, ovulatory dysfunction, hormonal imbalances, insulin resistance, and dyslipidemia (Bannigida *et al*., 2020). Disruptions in the normal communication between the hypothalamus, pituitary gland, and ovaries are believed to contribute to PCOS. We investigated the effects of adding metformin to Diane-35 on ovulation and ovarian energy metabolism in women with PCOS. PCOS, a prevalent reproductive illness, presents a complex

clinical picture involving hormone disruption and metabolic abnormalities. It is hypothesized that PCOS is a proatherogenic form of metabolic syndrome unique to women (Sanchez-Garrido & Tena-Sempere, 2020). Chronic low-grade inflammation is increasingly recognized as a key player in its development.

Insulin resistance is central to PCOS pathophysiology and is a hot topic in reproductive endocrinology. PCOS women often exhibit weight gain, elevated luteinizing hormone and testosterone levels, and higher HOMA-IR, with ovaries displaying multiple atretic follicles and large follicular cysts with poorly formed granulosa cells (Moghetti & Tosi, 2021). Diane-35, containing 2mg of cyproterone acetate and 35 micrograms of ethinyl estradiol, is used to treat PCOS patients with hyperandrogenism by activating the progesterone receptor and inhibiting androgens like testosterone. Metformin improves insulin sensitivity and glucose metabolism in PCOS women (Tosi *et al*., 2017). Combining Diane-35 with metformin improves hyperandrogenemia, insulin resistance, BMI, and luteinizing hormone levels, restoring reproductive functioning (Zhou *et al*., 2020).

After three treatments, significant changes in LH, FSH, and LH/FSH ratios were observed, with acne severity ratings reduced. Both therapy groups showed increased BMI, but the combined therapy group saw a more significant decrease in BMI and body fat percentage after three months (Dapas *et al*., 2020). Granulosa cells in PCOS women showed reduced proliferation and increased apoptosis, which improved with Diane-35 and metformin therapy (S. Zhang *et al*., 2020). PCOS patients have a higher prevalence of overweight and obesity, linked to insulin resistance and hyperandrogenism. Insulin resistance is a precursor to more severe reproductive and metabolic disorders (Stepto *et al*., 2019). Although oral contraceptives like Diane-35 do not significantly alter weight, combined therapy with Diane-35 and metformin reduced BMI after three months (Stepto *et al*., 2020).

PCOS patients often have elevated androgen activity, manifesting as acne and hirsutism. Diane-35 and metformin significantly reduced acne scores, though hirsutism symptoms remained largely unchanged (Gao, 2018). The impact of Diane-35 on lipid metabolism is controversial, but our research showed improved lipid metabolism and glucose metabolism with the combined therapy (Ziegenhain *et al*., 2017). PCOS patients exhibit altered energy metabolism in the ovaries, with reduced glucose consumption and disrupted glycolysis. Diane-35 and metformin combination therapy improved ovarian energy metabolism and follicular development (Xiong *et al*., 2019). Metformin therapy reduced endometrial hyperplasia by down-regulating PKM2, and the combined therapy modulated key mediators related to glycolysis and energy metabolism (Geng *et al*., 2023; Hu *et al*., 2018).

Conclusion

Our results showed that treating PCOS patients with Diane-35 and metformin reduced pathological current alterations. Additional research indicates that combining Diane-35 and metformin may enhance ovarian energy metabolism by controlling the glycolysis pathway. Mechanistic investigations in PCOS patients treated with Diane-35 with metformin suggested that the treatment's therapeutic benefits may be connected to the control of glycolysis-related mediators. The biological role of the mediators involved in glycolysis in PCOS patients needed more investigation. Ultimately, PCOS affects women of childbearing age and has negative consequences for fertility and conception by interfering with hormone levels and the timing of ovulation. Because of its complexity and diversity, assessing and diagnosing researchers hope this work will help medical professionals better care for PCOS patients by expanding their knowledge of the disease's pathophysiology.

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