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Association Of Diane-35 And Metformin, Alone And In Combination, On Hepato-Renal Profile In Un-Married Females With PCOS

Asma Ahmed[1*], Aiza Ashfaq[1], Farzana Shamim[1], Rehana Badar[1,3], Hamna Shahid[1], Ghazala Irshad[1,2], Samra Hafeez[1,4], Afaq Akram[1]

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. Metformin and Diane 35 are used in the treatment of polycystic ovaries. 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 prospective study aims to determine the effects of diane 35 and metformin on CBC, LFTS, RFTS, and LH in the blood of 500 polycystic ovarian syndrome un-married patients at Khawaja Muhammad Safdar medical college, Allama Iqbal Memorial Teaching Hospital Sialkot, Punjab Pakistan and The University of Lahore, Lahore by using non-probability convenience sampling technique and by dividing all patients in Group I (control group), GroupII (administered metformin), Group III (administered diane-35) and Group IIV (diane-35 and metformin) for three months. All groups' preliminary weight and biochemical tests had been measured. Statistically analysed results showed a vairation in the age group and BMI of patients (24.00±3.80 to 29.26±2.84 years and 22.02±3.70 and 27.08±5.96 Kg/m^2 respectively). In all groups there were 86.7% participants with a history of acne and 13^1.2% 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 group II, 1% participants were with multiple small follicles in both ovaries, 16.7% with multiple cysts, 60% with PCO. In group III, 13.3% participants were with a cyst in the ovary, 13.3% with cystic ovaries, 6.7% with multiple small follicles in both ovaries, 6.7% with multiple cysts, and 46.7% with PCO. In group IV, 26.7% participants were with cystic ovaries and 60% with PCOS. Results showed that treating PCOS women with diane-35 and metformin reduced pathological alterations. Ultimately, PCOS affects women of child bearing 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.*

1. Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore, Punjab, Islamic Republic of Pakistan
2. University College of Medicine and Dentistry, The University of Lahore, Lahore, Punjab, Islamic Republic of Pakistan
3. Department of Biological Sciences, Superior University, Lahore, Punjab, Islamic Republic of Pakistan
4. Shrif Medical and Dental College, Lahore, Punjab, Islamic Republic of Pakistan

* Corresponding author: asma.ahmed@imbb.uol.edu.pk

1.0 Introduction

Primary infertility is caused mostly by polycystic ovary syndrome (PCOS), a metabolic condition and endocrine disorder that affect 8-13% of reproductive-aged women worldwide. Hyperandrogenism (hirsutism, acne), menstrual irregularities, ovulatory dysfunction, an imbalance of reproductive endocrine hormones, insulin resistance, and dyslipidemia are all clinical manifestations of polycystic ovarian syndrome. Greater LH synthesis is favoured over higher follicle-stimulating hormone(FSH) secretion in women with PCOS because of increased gonadotropin-releasing hormone pulse frequency. However, ovarian follicular atresia and hyperandrogenemia are hallmarks of PCOS because of a relative shortage in FSH production, which often leads to delayed or halted follicular growth and diminished aromatase activity. Furthermore, elevated insulin levels in PCOS patients may stimulate LH expression and ovarian and adrenal androgen production. 14-18 % of reproductive-age women worldwide are afflicted with this disease. It has been shown that young women with PCOS have a fourfold increased risk of developing endometrial cancer compared to those without PCOS who do not have (Zhang et al., 2020).

The National Institutes of Health sponsored a definition conference in 1990. The criterias of PCOS were oligo-ovulation, clinical or biochemical hyperandrogenism, and the exclusion of other known disorders, including late-onset congenital adrenal hyperplasia and Cushing's syndrome (NIH). Two criteria must be satisfied to diagnose after alternative causes, including congenital adrenal hyperplasia, androgen-secreting tumors, and Cushing's disease, have been ruled out(Abdalla et al., 2021). Ultrasound evidence of PCOS includes an increased ovarian volume (>10 mL), the existence of 12 or more follicles (each measuring 2-9 mm in diameter), or both. This is the case, no matter the echogenicity of the ovarian stroma or the location of the follicles. One affected ovary is all that's needed to diagnose PCOS. Women with PCOS on sonography may have regular menstrual cycles while not showing any signs of hyperandrogenism in their clinical or biochemical profiles.

PCOS devastates the emotional and physical well-being of women who experience it. Metformin reduces insulin resistance in people with PCOS, and the combination of metformin plus insulin has become standard practice for treating PCOS. Historically, individuals with PCOS have been treated with a combination of ethinyl estradiol and cyproterone acetate, which can protect the endometrium, regulate the menstrual cycle, and reduce polytrichia and acne caused by hyperandrogenism. Bailing capsules are made from a low-temperature-fermented Cordyceps sinensis strain that lowers cholesterol and glucose levels, safe guard liver and kidney function, and regulate endocrine cooperation (Okamura et al., 2017). Recent research has shown that enhancing ovulation with diane-35 with a bailing capsule is more effective than with diane-35 alone. Hence some researchers recommend using a bailing capsule as an adjuvant treatment for PCOS (Hussein & Soslow, 2018).

Diane-35 contains cyproterone acetate, a progesterone that acts as an anti-male hormone by increasing testosterone activity in the skin and other tissues. Diane-35's ability to regulate hyperactive male hormones also means it may help with presenting skin issues like acne and excessive hair growth (Sanchez-Rangel & Inzucchi, 2017). Hormonal contraceptives, metformin, myoinositol, orlistat, and GLP1 agonists are only a few medications that can help with PCOS. Physician prescribe diane-35, which combines 35 g of ethinylestradiol and 2 mg of cyproterone acetate, to patients with PCOS and hyperandrogenism (Rena et al., 2017 and Zhang et al., 2020). Moreover, women with PCOS-IR can restore fertility and prevent further atypical endometrial hyperplasia with the help of metformin (N, N-dimethylbiguanide) and oral contraceptives(Wang et al., 2019).

Oral contraceptives pills (OCP), insulin sensitizers, cyclic progestins, anti-androgens, and fertility medicines are all used in PCOS pharmacotherapy (Kempegowda et al., 2020). Metformin is recommended for PCOS in some circumstances to improve fertility and to treat

menstrual irregularities in women unable to take OCPs, and to treat prediabetes or type 2 diabetes when lifestyle changes fail in the presence of both conditions (Jia et al., 2020). Activating the progesterone receptor and inhibiting testosterone's androgenic effects are the mechanisms of diane-35 and diane-35 with metformin may help patients with PCOS to lose weight, lower their LH, improve insulin resistance, and lessen hyperandrogenism (Garad et al., 2019), while their oestrous cycle and ovulation was recovered, which suggest that the combined treatment can address PCOS problems (De Santi et al., 2019 and Liu et al., 2022).

Metformin improves ovulation, decreases testosterone levels, and lengthens the time between periods in women. Metformin reduces insulin levels and modifies insulin's effects, which may be one of how it influences ovarian androgen production, the proliferation of theca cells, and the formation of the endometrium. Additionally, it inhibits ovarian gluconeogenesis, perhaps by a direct effect, which reduces ovarian androgen production(Zhang et al., 2022). Metformin is often started at 500 mg once daily with meals to establish tolerance. After the first week, the dose is increased to 1500 mg daily, then maintained at 1000 mg for another week. The typical daily dose is between 1500 and 2550 milligrams (500 or 850 mg thrice daily). One thousand milligrams daily is the typical dose at which noticeable clinical effects occur. Those with PCOS who do not react to metformin at a dosage of 1500 mg daily respond effectively to metformin at 2000 mg (Wolf et al., 2018). Women with native PCOS who took metformin had a higher ovulation rate than those who took a placebo. Still, the two treatments had no significant differenceregarding cumulative ovulation, pregnancy rates, or live births(Glueck & Goldenberg, 2019 and Fong et al., 2021).

High levels of androgens in the blood are linked to developing hirsutism and acne in PCOS patients. While preliminary evidence suggests that diane-35 helps alleviate acne for PCOS patients, more study is needed to identify the optimal duration of diane-35 treatment for hirsutism, acne, and other hyperandrogenism-related symptoms (Kakoly et al., 2018). Persson et al. (2018) studies show that after three cycles of treatment, the combination of diane-35 with metformin significantly reduces the symptoms of hirsutism and acne (incidence lowered by 60.8% and 72%) and a combination of diane-35 and metformin reduced acne severity in individuals with PCOS, although hirsutism scores did not improve. The precise mechanism of diane-35 alters lipid metabolism in the blood is unknown(Persson et al., 2021).

Osibogun et al. (2020) studies have shown that PCOS patients have higher TG, TC, and HDL-C levels. Combining diane-35 with metformin for three months improves lipid metabolism. After three months of treatment, PCOS patients using Diane-35 did not affect their degrees of insulin resistance. Still, following a previous study on sugar metabolism, they did have a little adverse effect on their fasting insulin levels. Fasting blood sugar, fasting insulin, insulin resistance index, and insulin sensitivity index are all unaffected by three months of Diane-35 treatment(Osibogun et al., 2020). Moreover, Teede et al. (2021) research showed that fasting glucose levels and HOMA-IR drastically decreased after three months of combination medication, suggesting that the combination treatment had a beneficial influence on glucose metabolism. Lastly, PCOS affects a woman's fertility and capacity to conceive by influencing her hormone levels and the onset of ovulation.

Materials and Methods

Study design and sampling technique

This was a prospective study, conducted at Khawaja Muhammad Safdar medical college and Allama Iqbal Memorial TeachingHospital Sialkot by using non-Probability convenient sampling method (Shrivastava & Jagdev, 2019). A sample size of 500 females was used, including 450 single (unmarried), 50 healthy women in the control group, and was determined

after taking the prevalence of PCOS as 40.9 % among females. The margin of error was considered 5% with 95% confidence interval.

Inclusion and exclusion criteria

Females19-48 years of age, clinicallyconfirmed (physical appearance, ultrasonography) and biochemically (LH and FSH ratio >:1:1 to 2:1 or 3:1) hyperandrogenism.Females with oligo or chronic anovulation and polycystic ovaries. Females who were individually suffering from hirsutism with no polycystic ovaries. Females who were diabetic patients and patients.Patients already have impaired liver or kidney function due to related diseases.

3.9 Procedure:

After attaining ethical approval and patient permission to participate in the study (Annexure I), the enrolled female patients were divided into four groups by randomization. Group I was the control group (healthy unmarried females), Group II was metformin administered, Group III was Diane-35 administered and Group IV was treated with a combination of Diane-35 and metformin for three months. All groups' preliminary weight and biochemical tests such as liver function test (LFT), Renal functioning Test (RFT), complete bloodcomplete(CBC) and fasting blood sugar was noted in addition to the ultrasonographical picture. A 5 c.c blood was withdrawn each time from the patient's pre and post-therapy. The serum was separated by centrifugation at 3000 rpm for 2 minutes and stored at -20^{o}C until analysis. Pre and post-treatment sampling was done. A well-structured questionnaire was also recorded, including the patient's clinical symptoms, dietary habits, food frequency chart, BMI, oligo-menorrhea or amenorrhea, and biochemical analytes results while blood was analyzed for the estimation of of serum Alanine transaminase (ALT), Aspartate transaminase (AST), total and free bilirubin, albumin and alkaline phosphatase (ALP), urea, creatinine and CBC (TLC, DLC, haemoglobin, PCV, MCV, MCH, MCHC and platelet count) via kit method, spectrophotometric and calorimetric assay.

Statistical analysis was performedby using One-way ANOVA through SPSS. A P value <0.05 was considered indicative of statistical significance.

Results

Age and BMI of study participants

In the group I, the mean age of the participants was 24.00±3.80 years. The minimum age was 20 years, and the maximum was 28 years.The mean age of the participants in the group II was 25.53±6.41 years. The minimum age was 14 years, and the maximum was 37 years. In the 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 the 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. In the group I, the mean BMI of the participants was 22.02±3.70.The mean BMI of the participants in the group II was 27.92±7.036,group III was 26.97±6.35, andgroup IV was 27.08±5.96 (Figure 1).

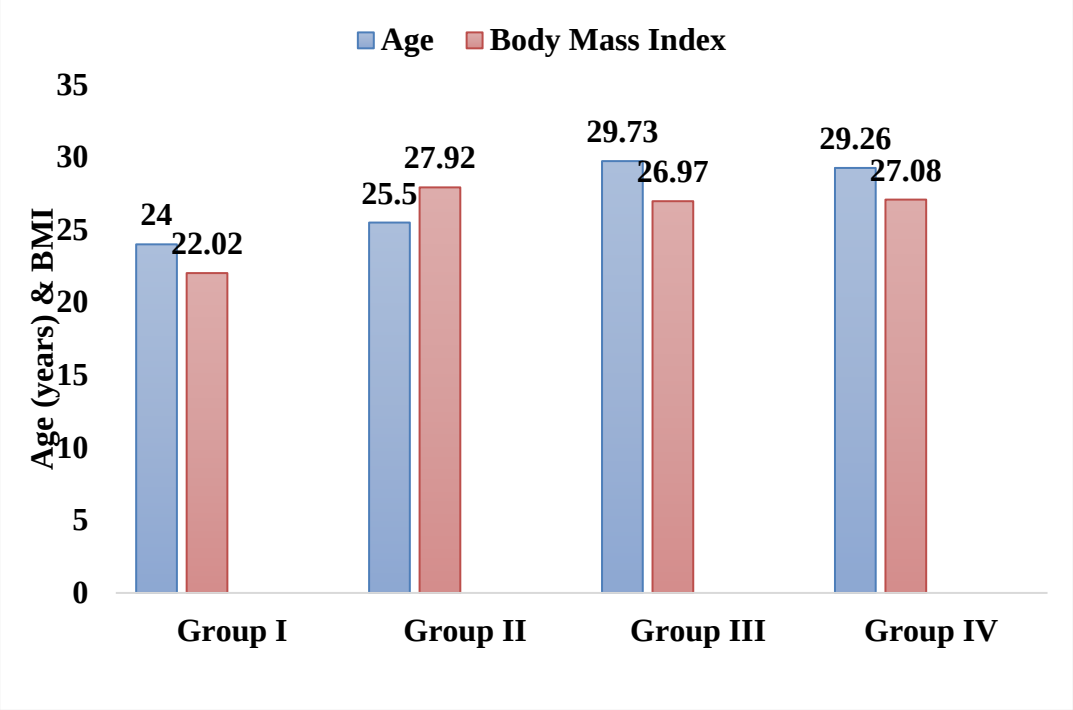

Figure 1 Age and BMI of study Participants in all groups

Occupation of study participants

In group I, there were 40% housewives, 0% school teacher, 60% students and 0% teachers. In group II, there were 33.3% housewives, 6.7% school teacher, 53.3% students and 6.7% teachers. In group III,there were 53.3% housewives, 0% school teacher, 33.3% students and 13.3% teachers. In group IV,there were 83.3% housewives, 0% school teacher, 33.3% students and 13.3% teachers (Figure 2).

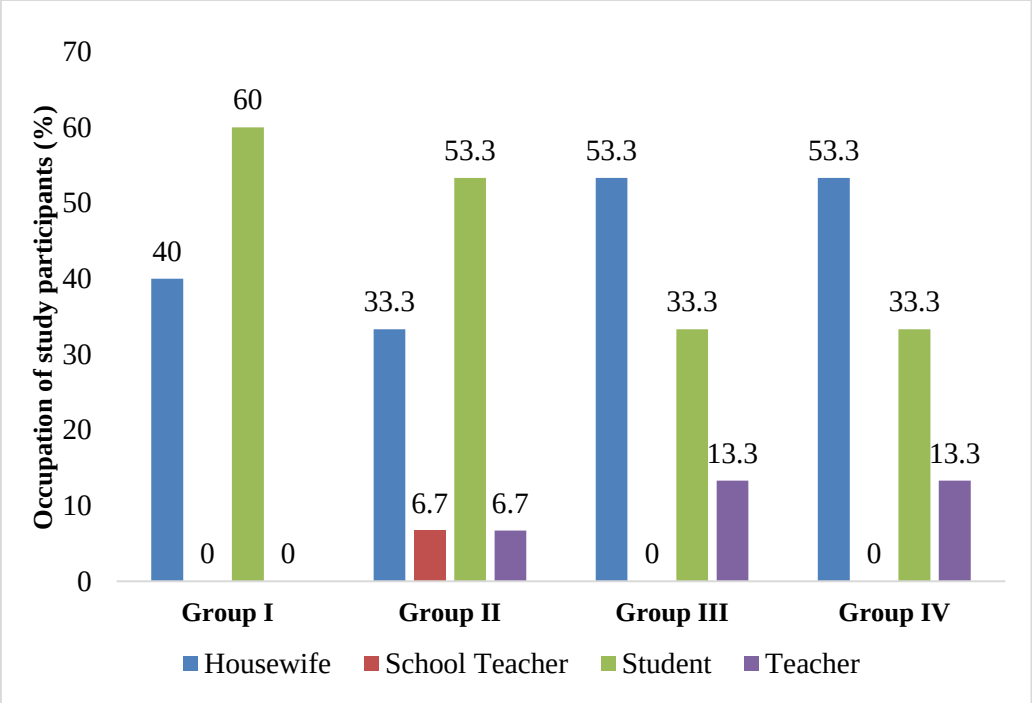

Figure 2: Occupation of study participant's percentage in different group

History of physiological parameters among study participants

In all the groups, 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 was no participant with depressed mode.In group II, III and IVthere were 93.3% participants with depressed mode and 6.7% without depressed mode. In group I there was no participant with sleep disturbance.In group 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 group 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 normal healthy group no participant with headache. In group I there was no participant with abdominal bloating.In group 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 group II, III and IV there were 13.3%, 20% and 20% participants respectively with history of diabetes (Figure 3).

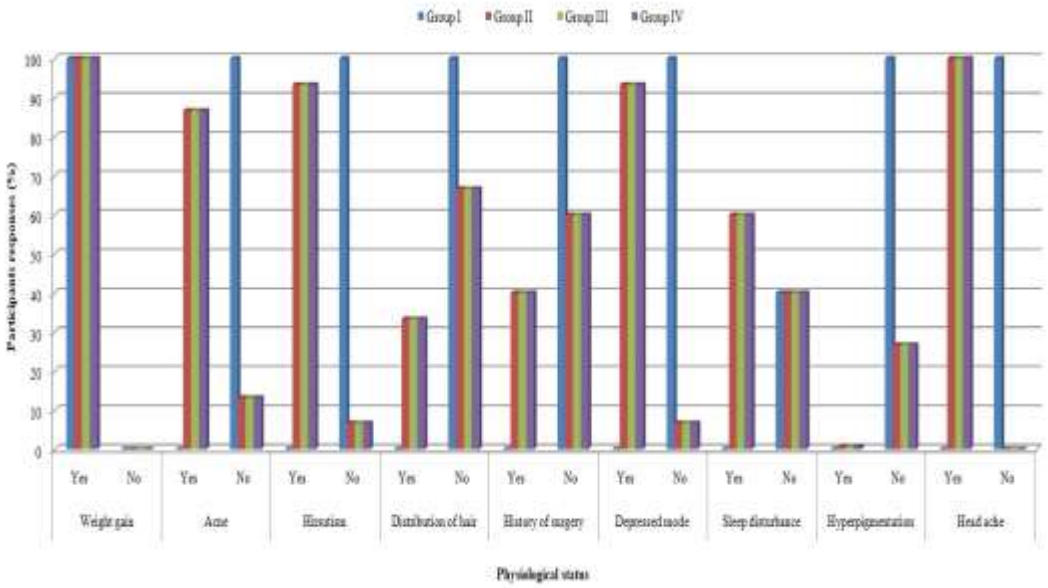

Figure 3: History of physiological parameters among study participants

Findings of ultrasonography

Results showed that participants in group II had maximum ratio of PCOs, followed by group III and IV, while cystic ovaries, cyst in ovaries were not shown in group I and II. Cystic ovaries were dominant in IV. Participants of Group I were clear in ultrasonographic finding (Figure 4).

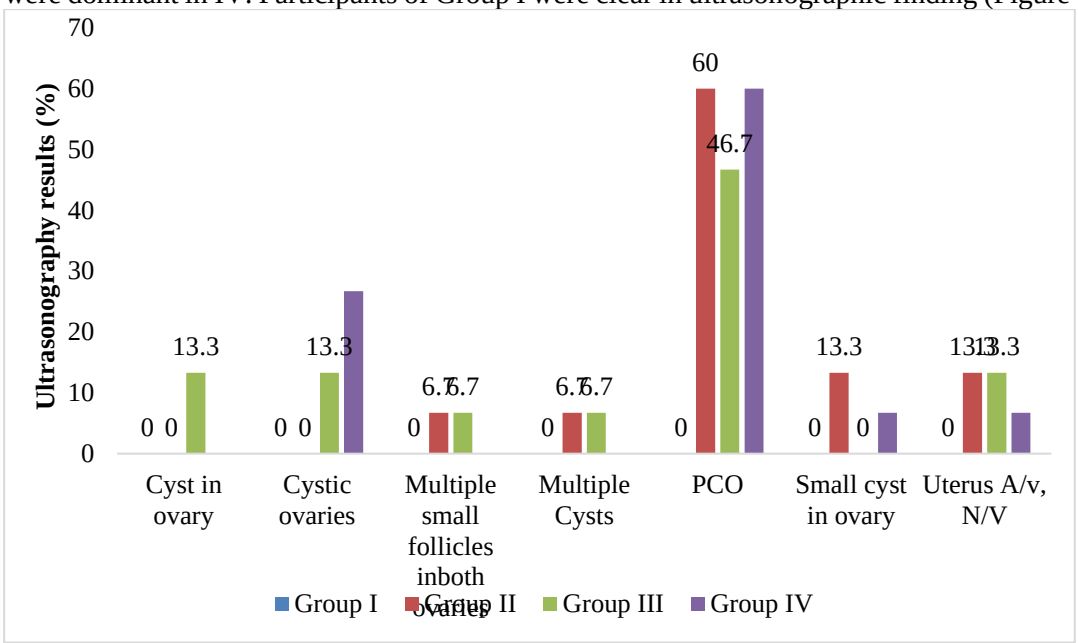

Figure 3 Ultrasonography findings (%) among participants

Level of biochemical markers in studygroups

There was no significant difference in the mean values of Follicular Stimulating Hormone in treatment groups (p-value:0.339). There was no significant difference in the mean values of Serum Luteinizing Hormone in treatment groups (p-value:0.063). There was significant difference in the mean values of AST level in treatment groups (p-value:0.000). There was

significant difference in the mean values of ALT level in treatment groups (p-value:0.000). There was significant difference in the mean values of Alkaline phosphatase level in treatment groups (p-value:0.000). There was significant difference in the mean values of TLC in treatment groups. (p-value:0.029). There was significant difference in the mean values of Haemoglobin 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 significant difference in the mean values of MCHC in treatment groups (p-value:0.99). There was 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 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) (Table 1)

Table 1: Levels of different markers after treatment with diane-35 and metformin in study groups

	Group I	**Group II**	**Group III**	**Group IV**	**p-value**
FSH	8.52±1.50	5.26±0.67	7.80±0.66	7.80±0.66	**0.339**
LH	14.00±1.35	6.97±1.10	10.76±2.80	10.76±1.80	**0.063**
ALT	20.40±1.14	28.61±2.19	53.20±5.44	41.00±3.84	**<0.001**
AST	22.80±2.58	31.26±3.31	72.93±2.91	33.20±4.34	**<0.001**
ALP	61.20±7.56	244.86±37.93	409.10±23.63	326.60±11.60	**<0.001**
TLC	7.0±1.58	7.59±1.04	9.86±1.00	10.68±1.84	**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±3.88	**0.167**
MCV	80.00±3.00	74.00±9.46	80.22±1.60	80.22±5.60	**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	310.6±28.58	286.26±24.60	247.33±22.35	234.4±17.79	**0.007**
Urea	26.0±4.18	21.66±2.32	50.73±3.09	49.93±4.96	**<0.001**
Creatinine	0.60±0.01	0.56±0.11	1.79±0.43	3.14±0.61	**<0.001**
Albumin	2.72±0.52	5.38±0.75	4.69±0.93	3.99±0.61	**0.43**

Discussion

PCOS is the leading cause of primary infertility in reproductive-aged women and is a complicated endocrine disorder and metabolic illness that affects 8-13% of women worldwide.

Hyperandrogenism (hirsutism, acne), menstrual irregularity, ovulatory dysfunction, reproductive endocrine hormone imbalance, insulin resistance, and dyslipidemia are all clinical symptoms of polycystic ovary syndrome(Bannigida et al., 2020). It has long been believed that PCOS results from disrupting the normal communication between the hypothalamus, pituitary gland, and ovaries. One of the most prevalent reproductive illnesses, PCOS, has a complex clinical picture that includes hormone disruption and other metabolic abnormalities. It has been hypothesized that PCOS is a proatherogenic form of metabolic syndrome unique to women(Sanchez-Garrido & Tena-Sempere, 2020). There is still a lack of understanding about what causes this disease, but it is becoming clearer that chronic low-grade inflammation is a key player in its development.

Diane-35, which consists of 2mg of cyproterone acetate and 35 micrograms of ethinyl estradiol, is often used to treat PCOS patients with hyperandrogenism. It achieves its effects by activating the progesterone receptor while simultaneously inhibiting the actions of androgens like testosterone. It is well known that metformin helps PCOS women by adjusting their insulin sensitivity and glucose metabolism. The therapy of PCOS patients with diane-35 and metformin has been shown to improve their hyperandrogenemia and insulin resistance, reduce their BMI, and lower luteinizing hormone levels. Diane-35 was proven beneficial in restoring reproductive functioning in dihydrotestosterone-induced PCOS patients and a combination of diane-35 and metformin therapy relieved PCOS problems, including lowered body weight, decreased testosterone and luteinizing hormone levels, and restored oestrous and oestrous cycles and ovulation in the PCOS (Zhou et al., 2020).

Following three treatments, LH, FSH, and LH/FSH had dramatically altered within the same treated group. However, there was no noticeable variation between the two therapy groups. Acne severity ratings clearly reduced before and after some therapy, and this trend was also seen for other hormonally linked parameters. The two groups had no discernible difference. Three months into therapy, both groups showed increases in BMI. The body mass index of the combined therapy group decreased significantly more than that of the Diane-35 alone treatment group. Following a three-month course of combo therapy, there was also a statistically significant reduction in body fat percentage ($p<0.02$). There was no statistically significant difference seen. Other body markers used in this investigation showed no significant changes(Dapas et al., 2020).

Granulosa cells in the ovarian tissues of PCOS women both proliferate less (as measured by PCNA expression) and undergo more apoptosis (as measured by the TUNEL test). Research has shown that LH might hinder follicle formation and development by inhibiting granulosa cell proliferation. In testosterone-induced PCOS human chorionic gonadotropin (hCG) plus l-norgestrel-induced PCOS, granulosa cells in the ovaries show decreased cell proliferation with degenerated granulosa cell layers, and an increased number of TUNEL granulosa cells were found in dehydroepiandrosterone-induced PCOS(Laven, 2019).

PCOS patients have a high risk of complications, including infertility and menstrual irregularities, but few studies have proven effective therapy for these issues. Over time, how PCOS is treated will evolve in response to patients' feedback on the most bothersome symptoms. Many women feel that oral contraceptives promote weight gain and consider this one of the worst side effects of these medications. Many women choose not to take oral hormonal contraception because they are concerned about their physical appearance. It is also found that after 3 months of therapy with diane-35, in addition to metformin, the patient's body mass index (BMI) reduced (Stepto et al., 2020).

PCOS patients often have elevated androgen activity in the blood, which manifests physically as acne and hirsutism. Although prior research has shown that diane-35 may reduce acne in PCOS patients, the length of time it takes for hirsutism, acne, and other hyperandrogenic symptoms to resolve is still unknown (Thomas et al., 2019). According to

certain research, acne and hirsutism symptoms may be greatly reduced following 3 cycles of therapy with diane-35 and Metformin (incidence fell 60.8% and 72%, respectively). Three months into therapy, our research showed that PCOS individuals using diane-35 in conjunction with etformin had improvements in their acne scores. In contrast to acne scores, hirsutism symptoms did not alter much (Efremova et al., 2020).

Follicle growth requires a steady supply of energy, provided by granulosa cells' absorption of glucose from neighbouring tissues through the GLUT on their cell membranes and the subsequent synthesis of pyruvate and lactic acid via the glycolysis process. Researchers observed that the rate of lactic acid synthesis in the follicular fluid rose dramatically as the follicle's diameter grew, indicating that glycolytic activity was elevated during follicle development. In addition, follicular fluid from PCOS patients contained far less lactic acid than healthy women. Reversing PCOS in patients required combined therapy with diane-35 and metformin. In human investigations, diane-35 was shown to raise triglyceride levels without significant adverse effects on metabolism in PCOS patients, and diane-35, combined with metformin, improved metabolic profiles in this patient population. Diane-35 with metformin might enhance ovarian energy metabolism by controlling glycolysis(Xiong et al., 2019).

Metformin therapy reduced endometrial hyperplasia in PCOS patients by down-regulating Pyruvate kinase isozymes M2 (PKM2) (Hu et al., 2018). PKM2 was down-regulated in the ovarian tissues of PCOS patients, which was mitigated by the diane-35 plus metformin therapy. The findings imply that PKM2 plays a crucial role in the glycolysis of follicular energy metabolism and that diane-35 with metformin might ameliorate the PCOS glycolysis process. Studies have indicated that the expression of LDH-A in granulosa cells is greater than in oocytes. The expression of Lactate dehydrogenase A (LDH-A) in Combined oral contraceptives (COCs) was dramatically elevated in the late stage of follicular development(Yang et al., 2021). It has been hypothesized that follicular developmental abnormalities in PCOS might be linked to lactic acid generation and that androgen could block LDH-A expression to diminish this production(Hu, Li, et al., 2018). However, in PCOS patients, an overabundance of nerve growth factors in the follicular fluid may drastically lower LDH-A expression, disrupt communication between granulosa cells and oocytes, and ultimately stunt oocyte development. Metformin therapy might raise the LDH-A expression in the endometrial tissues of PCOS patients with hyperplasia.

Furthermore, the connection between LDH-A and Sirtuin 1 (SIRT1) might enhance metabolic channelling and the consequent epigenetic alteration in the nucleus (Tao et al., 2015). Moreover, recent studies found that SIRT1 expression was significantly reduced in the ovaries of PCOS patients, which could be improved by metformin treatment. Similarly, our data showed that SIRT1 was down-regulated in the ovary of POCS patients and that this down-regulation was restored by the diane-35 plus metformin treatment. Collectively, the therapeutic benefits of diane-35 with metformin therapy in the PCOS patients may be connected with the control of glycolysis-related mediators (Zhang et al., 2020).

The present study demonstrated the beneficial effects of diane-35 plus metformin treatment in PCOS patients for the first time. More importantly, mechanistic studies revealed that several key mediators related to glycolysis and energy metabolism could be modulated by the Diane-35 plus metformin treatment. However, this study is still in its early stages, and further research into the functional roles of these mediators is required. Further clinical investigations may be conducted to establish the expression of these critical mediators in PCOS patients following therapy with Diane-35 and metformin and to evaluate the clinical importance of these mediators in the context of PCOS.

Summary

This research was conducted at Khawaja Muhammad Safdar medical college and Allama Iqbal Memorial Teaching Hospital Sialkot. All the selected patients were screened at The University

of Lahore. The mean age of the participants in the metformin treated group was 25.53±6.41 years while BMI of participants in this group was 27.92±7.036 Kg/m^2 as compared to diane-35 treated group (BMI was 26.97±6.35 Kg/m^2), metformin+ diane-35 treated group (BMI was 27.08±5.96 Kg/m^2) and healthy group (BMI was 22.02±3.70 Kg/m^2). In all experimental groups, 86.7% participants were with a history of acne., 93.3% participants were with a history of hirsutism, 33.3% participants were with a distribution of hair; 93.3% participants were in depressed mode, 60% participants were with sleep disturbance, 73.3% participants were with hyperpigmentation, 100% participants were with a headache, more than 73.3% participants had abdominal bloating, 40% participants were with a history of surgery and almost 20% participants were with a history of diabetes. There was no significant difference in the mean values of the follicular stimulating hormone and serum luteinizing hormone in treatment groups. There was a significant difference in the mean values of haemoglobin and TLC levels in treatment groups. There was no significant difference in the treatment groups' mean values of MCH, MCV, MCHC, platelet count, urea and creatinine. There was a significant difference in the mean values of AST and level in treatment groups.

Current results showed that treating PCOS women with Diane-35 and metformin reduced pathological alterations. Further research is needed, but preliminary results show that Diane-35 and metformin, when combined, may enhance ovarian energy metabolism by controlling the glycolysis pathway. Mechanistic investigations suggested that Diane-35 with metformin's therapeutic actions in PCOS patients may be linked to the modulation of glycolysis-related mediators.

Conclusion

Current 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 diagnosingresearchers 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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