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Research Article

Effect of Choline Inositol and Metformin Combination vs Metformin Alone on Ovarian and Incertain: A Cross Sectional Study

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KEY WORDS:

Choline inositol
Metformin
Glp1
PCOS
FSH
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LDL
HDL
Triglyceride

Abstract:

Polycystic ovary syndrome (PCOS) is a widespread ordinary disorder that affects adult females. PCOS is causing hirsutism, menstrual abnormalities and infertility, PCOS is related with several conditions of health, such as resistance of insulin, metabolic syndrome, the high risk of cardiovascular infirmity, pregnancy-related involvement, and psychological issues PCOS, So management encompasses a range of strategies and treatments. In recent years, a lot of researcher have been focused on analyze the probable outcome of inositol supplements and make proposition that choline inositol are potent substitutional for metformin in treating PCOS but the results of various literatures contained sufficient controversy, to re-evaluate the use of these two agents in the current study. The current study aims to determine the effect of choline inositol and metformin combination vs metformin alone on ovarian and incertain: A cross sectional study. This study was performed at the Maternity and Pediatrics Teaching Hospital in ALdiwaniyah Province, Iraq. The study involved a total of (150) patients at (18-<40) years. A gynecologist uses Rotterdam criteria and ultrasound ovarian morphology in the selection of PCOS patients. The study consisted of several groups: G1 is a thirty (n = 30) women had received oral Metformin 500 mg bid during meal for last three months and served as metformin group. G2 is a thirty (n = 30) women had received choline inositol supplement 500-500 mg per-oral twice daily for last three months and served as the choline inositol supplement group. G3 is a thirty (n = 30) women had received choline inositol and metformin combination, they received with the same above dose for last three months and served as the choline inositol and metformin combination group. G4 is a thirty (n = 30) women who have not received any treatment for PCOS and served as new diagnosis PCOS group. Finally G5 is a thirty (n = 30) healthy women control group (control group). Characteristics after 3 months included body mass index, type of period (regular, irregular ,amenorrhea) and clinical features of high androgen (acne and hirsutism) were evaluated. The blood samples were 10 ml and then divided into two tubes. The first tube was left in the lab for 15 minutes and then separated at 3000 rpm for 8 minutes to measure follicular stimulating hormone (FSH), Luteinizing hormone(LH) , prolactin, TSH, lipid profile (HDL-cholesterol, triglyceride, total cholesterol, LDL) and HbA1c level. The second tube is separated for 20 minutes by centrifuge at 3000 rpm to estimate the incertain level. There was no significant difference in mean age. There was a significant difference in BMI and the order of groups based on the LSD test was as follows: G4, G2 followed by G1, then G3 and G5. There was a significant difference in rate of amenorrhea from the groups under the study and the order of groups based on the LSD test was as follows: G4-G1 followed by G2 then G3 and G5. There was also a significant difference in rate of cycle irregularity among the groups under the study and the order was as follows: G4-G1 followed by G2 then G3-G5. There was a significant difference in mean FSH and the order of groups based on LSD test was as follows: G3-G1 followed by G4 then by G2-G5. There was a significant difference in mean LH and the order of groups based on LSD test was as follows: G1 and G4 followed by G2-G3-G5. There was a significant difference in mean HDL and the order of groups based on LSD test was as follows: G3-G5 followed by the rest of groups. There was a significant difference in mean triglyceride and the order of groups based on LSD test was as follows: G4-G1 followed by G2 then by G3-G5. There was a significant difference in mean LDL and the order of groups based on LSD test was as follows: G4-G2 followed by G1then by G3-G5. There was a significant difference in mean serum GLP-1 and the order of groups based on LSD test was as follows: G5-G3 followed by G2 then by G4-G1.There was a significant difference in mean follicle volume and the order groups based on LSD test was as follows: G5-G3-G2-G1-G4. we could conclude that the administration of Choline inositol and metformin together causes an increase HDL level, FSH level,GLP1 level and improve amenorrhea, irregularity of cycle and follicle volume as compared with alone Choline inositol administration or alone metformin administration and control group. This combination above are efficient in reducing BMI, LH level, triglyceride level, LDL level compared to healthy individuals.

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INTRODUCTION

Polycystic ovarian syndrome (PCOS) is by far the most predominate metabolic ailment affecting female during their reproductive life. The disease in common among Iraqi women and it is one of the principal causes of infertility[1].

It is characterized by uneven menstruation, hyperandrogenism and polycystic ovarian morphology[2]. In addition, PCOS is related with several conditions of health, such as resistance of insulin, metabolic syndrome, the high hazard of cardiovascular ailment, pregnancy-related issues and psychological problems[3]. PCOS management encompasses a range of strategies and treatments. Lifestyle factors including exercise and diet changes have been shown to manage symptoms of PCOS and are among the first line of treatment . These changes can lead to weight loss, which has been shown to reduce PCOS characteristics, including insulin sensitivity and irregular menses[4]. Medications acting on hormones, including estero-progestin and insulin sensitizer, commonly treat PCOS symptoms[5]. As an iguanid insulin sensitizer, metformin may enhance insulin action without affecting insulin secretion. This medication is used as a first-line therapy for type 2 diabetes. Insulin resistance and hyperinsulinemia-metabolic traits shared by fatty female with PCOS and T2DM-are common in these cases. Metformin may help PCOS patients with endocrine issues, control ovarian function and lose weight[6].

Choline becomes low level in PCOS[7]. Choline might have an overarching role in regulating ovarian function[8]. New studies showed the potential effects of inositol supplementation for treating PCOS[9]. They invariably impress menstrual cycle regularity, carbohydrate metabolism and hyperandrogenism's clinical and laboratory symptoms. Inositol supplements also had been role in the reduction of triglycerides and LDL-cholesterol levels, but it did not have a great effect on HDL-cholesterol levels on patients that having metabolic diseases. multilateral endurable studies concerning with the trace of inositol supplements on lipid profiles in patients with metabolic diseases are important[10]. Choline and Inositol demonstrated that obese and non-obese PCOS women can benefit from the combination of insulin sensitizes. Choline and Inositol, combined with metformin, enhance overweight and the Clinical Picture of PCOS[11]. It also found that choline and inositol lowered LH but had no effect on FSH[12].

The prejudices of the present work are to determine the effect of choline inositol and metformin on Serum incertain(GLP1) and ovarian activities in Iraqi women with PCOS.

MATERIALS AND METHODS

Patients: The present study have been included a group of 150 patients with an average range of age from 18 to less than 40 years that they were selected randomly. Patients have been selected during the interval of performing this work. This work require the volunteering of 120 female from the population whom fulfilled the criteria of PCOS and 30 as a well healthy female. A gynecologist uses Rotterdam criteria and ultrasound ovarian features in the selection PCOS patients.

Inclusion Criteria of Patients:

- Patients with Polycystic ovary syndrome selected according to Rotterdam criteria and ultrasound.
- Age range of 18 to less than 40 years.
- Healthy women who have regular menstrual cycles and do not underlying endocrine disorder like hypothyroidism or DM.
- Patients received metformin or choline inositol alone or metformin and choline inositol together for previous 3 months, but not more than 6 months.
- Patients don't received any other drugs except metformin and choline inositol.

Exclusion Criteria:

- Age less than 18 years or equal and more than 40 years.
- Co-morbidities such as DM, endocrine disease, hyperprolactinemia, renal disease, dyslipidemia.
- Utilize of therapy for inhibition of resistance of insulin for hypertension, dyslipidemia and hyperglycemia.

Data Collection and Research Tool: patients with Polycystic ovary syndrome (PCOS) will be selected from the Maternity and Pediatrics Teaching Hospital in ALdiwaniyah Province, Iraq who interviewed by a specially prepared questionnaire for this study that includes demographic data and clinical data.

Grouping of Patients: Patients Were Categorized into Five Groups:

- **(G1):** Metformin group were received 500 mg orally twice daily with meal for the last three months.
- **(G2):** Choline inositol group were received Choline 500 and inositol 500 mg per oral twice daily with meal for the last three months.

- **(G3):** Choline inositol and metformin together group were received 500 mg orally twice daily with meal of metformin and choline inositol supplement was received 500/500 orally twice daily with meal for the last three months.
- **(G4):** New diagnosis (PCOS group) for those who have not received any treatment for PCOS, a gynecologist uses Rotterdam criteria and ultrasonic ovarian morphology in selecting PCOS patients.
- **(G5):** A healthy women (control group) were selected among the women with regular menstrual cycles every (21-35 days), without endocrine disorder androgen excess and have standard ovary shape.

Ethical Consideration: Verbal consent were taken from each patient before sampling specimens. This study was approved by the committee of publication ethics at College of Medicine, University of AL-Qadisiyah, Iraq (approval reference number No:301104 on 8 January 2024).

Time and Location of the Study: The patients were recruited from the Maternity and Pediatrics Teaching Hospital in ALdiwaniyah Province, Iraq. The study is dated back to September 2023 and extended to March 2024.

The Used Kits: Based on our results, the kits used in the current work are illustrated in (Table 1) and their origin.

Study Design: This a cross-sectional study was performed at the Maternity and Pediatrics Teaching Hospital in ALdiwaniyah Province, Iraq included total of 150 women (with a range of ages from from 18-<40 years). Recruitment of patients and procedures including PCOS and healthy women and biochemical and clinical characteristics after 3 months of treatment.

Sample Size Estimation: The size of the Sample's estimation was based on the following equation:

$$\text{Minima Sample Size } (n) = pX(1-p)XZ2/Me2$$

Where n is the minimal size of the sample size and **p** is the preponderance of polycystic ovary syndrome, according to[13], as high as 20 % among women within the fertile age. The Z express the Z-score at a 95 % confidence interval, which equals 1.96. Me express the inferior error accepted according to[14] as 0.05. Thus, the minimum size of samples was estimated to be about 246., however, because of the limitations of Time and resources and the lack of patient compliance, the study enrolled only 150 patients.

Anthropometric Assessment: It include both of age, weight (kg) and height (m). The following formula was used to get the BMI value: BMI is calculated as weight (kg) divided by height (m2)[15].

Blood Samples Collection: Instructions to the laboratory were delivered to the participants in the morning on the second day of menstruation and after a 10-hour fast. A disposable syringe was used to extract around 10 milliliters of blood, then separated into two tubes. After 15 minutes of waiting following blood collection, the serum in the first tube was centrifuged for 8 minutes at 3000 rpm and the resulting serum samples were ready to tested HbA1c, lipid profile (HDL-cholesterol, Triglyceride, Cholesterol), hormonal profile(FSH, LH). The sample of the blood in the second tube have been kept at 23 C° for 2 hours. After that, the serum has been separated utilizing a centrifuge running at 3000 rpm for 20 minutes. The collected serum from the second tube was placed in a deep freeze (-80°C) until the ELISA method could be used to analyze the serum for GLP1.

Elisa Method: This technique was used to measure GLP1 with ELISA kits.

Principle: The kits are Sandwich enzyme-linked immunosorbents used to determine GLP1 in the study groups quantitatively.

The Steps of the Procedure:

- All of reagents has been Prepared a standard solutions and samples according to the instruction. Bringing all of reagents at room temperature before the time of usage. It performed at room temperature.
- The number of strips required have been determined for the assay and the strips in the frames had been inserted in the frames for use. The unused strips should be stored at 2-8 C°.
- a 50µl of standard volume has been added to standard well. Take under account that we never combine biotinylated antibody to the standard well for the reason that the standard solution have biotinylated antibody.
- a volume 40µl of a volume of a specimen has been

combined to the sample wells after that combined 10µl of volume of anti-GLP-1 antibody to sample wells, after that step 50µl of streptavidin-HRP has been added to sample wells and standard wells (Not blank control well). Mix well. Cover the plate with a sealer and Incubated for one hour at 37°C.

- The sealer removed and the plate clean by washing five times with wash buffer. Soak wells with 300 µL wash buffer for 30 seconds to 1 minute per wash. Blot the plate onto paper towels or other absorbent material.
- 50µl substrate solution combined to each well and then added 50µl substrate solution B to each well. The plate Incubated and wrapped with unused sealer for 10 minutes at 37°C in the dark.
- 50µl of volume of the Stop Solution combined to each well, the blue color will turn to yellow one at once .
- The optical density (OD value) of each well has been calculated by utilizing a micro plate reader set to 450 nm within 10 minutes after adding the stopping solution.

Calculation of Result: The results of ELISA have been studied considering the rate of match optical density readings for each standard and sample (OD). Then making a standard curve by drawing the mean OD value For each standard on the y-axis, compare the concentrations on the x-axis and determine the line of best fit.

VIDAS Method: This technique was used to determine the serum levels of the followings : Follicle Stimulating Hormone (FSH), luteinizing hormone (LH), thyroid stimulating hormone (TSH), prolactin. The assays were achieved according to instructions of manufactures kits of hormonal profile.

Bio-Rad ion-Exchange HPLC Method: Bio-Rad ion-exchange high performance liquid chromatography (HPLC) is the gold standard for HbA1c testing is distinct technology/method used in the Diabetes Control and Complications Trial (DCCT) and the UK Prospective Diabetes Study (UKPDS). To this day, healthcare providers around the world still follow the HbA1c testing guidelines, established by these landmark trials, to aid in the diagnosis and monitoring of patients living with diabetes.

Lipid Profile Assay: This biochemical assay for the quantitative determination of serum triglycerides (TG), total cholesterol (TC), high density lipoprotein (HDL). The assays were achieved with Beckman Coulter AU analyzers according to instructions of manufactures kits of lipid profile except low-density lipoprotein (LDL) was calculated using the Freetailed equation according to (Dhafer A F. *et al* 2019) LDL = (TC-HDL-[TG/5]).

Statistical Analysis: Data have been collected, epitomized, outline and presented utilizing statistical package for social sciences (SPSS) version 23 and Microsoft Office Excel 2010. Qualitative (categorical) variables were expressed as number and percentage, whereas, quantitative (numeric) variables were first evaluated for normality distribution using Kolmogorov-Smirnov test and then accordingly normally distributed numeric variables were expressed as mean (an index of central tendency) and standard deviation (an index of dispersion) and range.

The following statistical tests have been used:

- One way analysis of variance (ANOVA) was utilized to calculate the difference in mean of numeric variables among more than two groups as long as that these numeric variables were normally distributed. One way ANOVA has been followed by pos hoc least significant difference (LSD) test to find out individual differences in the mean values between any two groups among all of the groups.
- Pearson correlation test was find bi-variate correlation coefficient between any two variables and the corresponding level of significance. The level of significance was taken into account at p-value of equal or less than 0.05.

RESULTS AND DISCUSSIONS

Demographic Characteristics of Patients and Control Subjects: Demographic features of patients and control subjects are illustrated in (Table 2). There was no significant difference in mean age (p = 0.255).

There was a significant difference in mean BMI ($p<0.001$) and the order of groups based on LSD test was as follows: Newly diagnosed groups followed by choline inositol followed by metformin group then by choline inositol + metformin group and lastly by control.

Clinical Characteristics of Patients Enrolled in this Study: Clinical characteristics of patients involved in this works are shown in (Table 3). There was significant difference in rate of amenorrhea among study groups and the order was as follows: highest rate was reported

in newly diagnosed group followed by metformin group then by choline inositol group then by choline inositol + metformin and lastly by control group. There was also a significant difference in rate of cycle irregularity among study groups and the order was as follows: highest rate of irregular cycle was reported in newly diagnosed group followed by metformin group then by choline inositol group then by choline inositol + metformin and lastly by control group.

Rapprochement of Serum Hormonal Grade Between Study Groups: Rapprochement of serum hormonal grade between study groups is illustrated in the (Table 4).

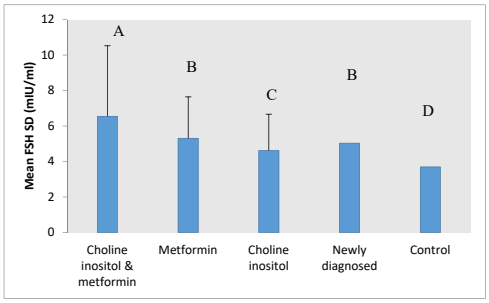

Fig. 1: Bra chart illustrating Rapprochement of serum FSH level between study groups

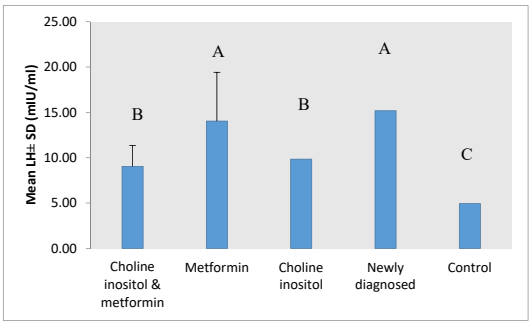

Fig. 2: Bra chart illustrating Rapprochement of serum LH level between study groups

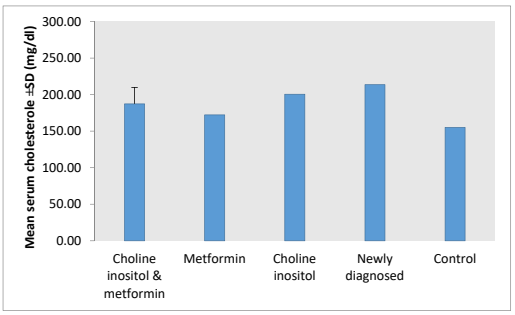

Fig. 3: Bra chart showing Rapprochement of serum cholesterol level between study groups

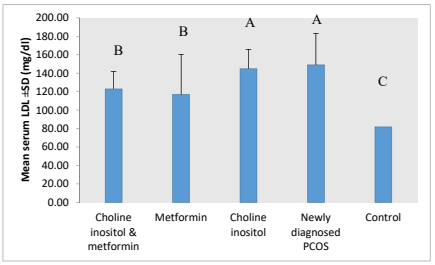

Fig. 4: Bra chart showing Rapprochement of serum triglyceride level between study groups

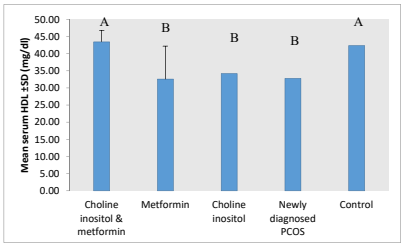

Fig. 5: Bra chart illustrating Rapprochement of serum HDL level between study groups

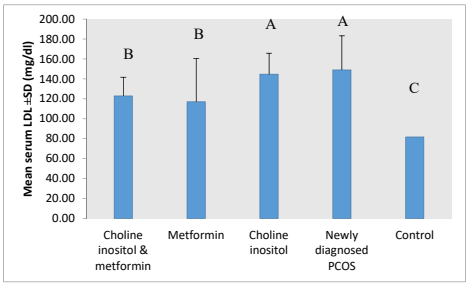

Fig. 6: Bra chart illustrating Rapprochement of serum LDL level between study groups

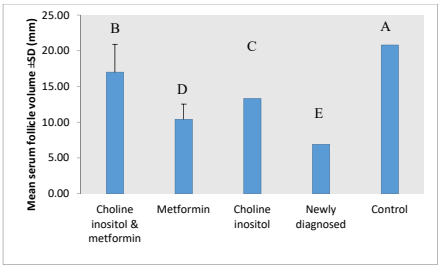

Fig. 7: Bra chart showing Rapprochement of follicle volume between groups of the study

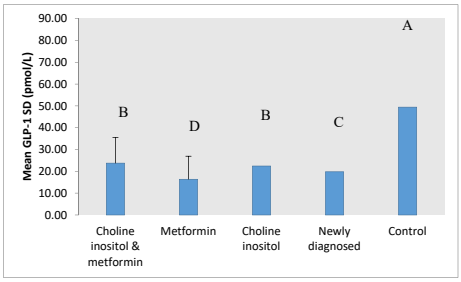

Fig. 8: Bra chart showing comparison of serum GLP-1 among study groups

Table 1: The Biomarker and biochemical kits used in this study with their companies and countries of origin

Rank	Kit	Company	Country
1	Human Glucagon-like peptide 1 ELISA Kit (Incertain hormone)	BT-LAB	China
2	FSH kit	bioMérieux	France
3	LH kit	bioMérieux	France
4	HDL-cholesterol kit	Beckman Coulter	USA
5	Triglyceride Kit	Beckman Coulter	USA
6	Cholesterol Kit	Beckman Coulter	USA
7	HbA1c Kit	Bio-Rad	USA
8	TSH Kit	bioMérieux	France
9	Prolactin Kit	bioMérieux	France

Table 2: Demographic characteristics of patients and control subjects

Characteristic	Choline inositol and metformin n = 30	Metformin Group n = 30	Choline inositol Group n = 30	Newly PCOS n = 30	Normal Control n = 30	p
Age (years)						
Mean ±SD	27.20 ±6.28	26.10 ±4.03	27.10±4.07	26.00±6.64	27.67±4.73	0.255 O NS
Range	18-39	19-31	20-33	18-35	23-37	
BMI (Kg/m2)						
Mean ±SD	24.45±2.73	27.86±4.49	28.11±3.24	29.40±5.29	21.92±2.37	<0.001 O ***
Range	19.53-28.6	21.76-35.16	22.66-34.29	21.78-39.06	17.4-26.22	

BMI: body mass index., SD: standard deviation., n: number of cases., O: one way ANOVA., **: significant at p = 0.01., ***: significant at p = 0.001

Table 3: Clinical characteristics of patients enrolled in this study

Characteristic	Choline inositol & metformin n = 30	Metform Group n = 30	Choline inositol Group n = 30	Newly PCOS n = 30	Normal Control n = 30	p
Amenorrhea						
Positive	3 (10.0 %)	9 (30.0 %)	6 (20.0 %)	18 (60.0 %)	0 (0.0 %)	<0.001 C*
Negative	27 (90.0 %)	21 (70.0 %)	24 (80.0 %)	12 (40.0 %)	30 (100.0 %)	
Cycle						
Irregular	9 (30.0 %)	18 (60.0 %)	16 (53.3 %)	30 (100.0 %)	0 (0.0 %)	<0.001 C*
Regular	21 (70.0 %)	12 (40.0 %)	14 (46.7 %)	0 (0.0 %)	30 (100.0 %)	

C: chi-square test., *: significant at p = 0.05., *: significant at p = 0.01

Table 4: Rapprochement of serum hormonal levels between study groups

Characteristic	Choline inositol & metformin n = 30	Metformin Group n = 30	Choline inositol Group n = 30	Newly PCOS n = 30	Normal Control n = 30	p
FSH (mIU/ml)						
Mean±SD	6.55±3.97	5.31±2.33	4.62±2.05	5.04±2.06	3.70±1.26	0.001 O ***
Range	3.39-17.3	1.63-8.21	2.1-9.1	2.94-8.76	1.53-5.72	
LH (mIL/ml)						
Mean±SD	9.04±2.32	14.05±5.38	9.84±2.70	15.18±3.89	4.93±1.67	<0.001 O ***
Range	6.8-13.4	8.5-25.5	6.53-15.8	9-21.22	2.5-6.9	

FSH: follicle stimulating hormone., LH: luteinizing hormone., n: number of cases; O: one way ANOVA., SD: standard deviation., **: significant at p = 0.01., ***: significant at p = 0.001

Table 5: Rapprochement of serum lipid profile between study groups

Characteristic	Choline inositol & metformin n = 30	Metformin Group n = 30	Choline inositol Group n = 30	Newly PCOS n = 30	Normal Control n = 30	p
Cholesterol (mg/dl)						
Mean±SD	187.35±22.26	172.30±34.58	200.50±24.22	213.60±36.86	155.13±20.54	<0.001 O ***
Range	162-237	115-236	165-236	137-267	123-188	
Triglyceride (mg/dl)						
Mean±SD	105.06±49.04	113.59±45.65	107.94±34.37	159.16±45.15	86.29±28.96	<0.001 O ***
Range	68.3-226	71.4-213	69.7-189	74.2-224	62.1-168	
HDL						
Mean±SD	43.44±3.39	32.60±9.61	34.19±4.10	32.79±3.70	42.37±2.49	<0.001 O ***
Range	38.1-48.3	7.1-39.2	26.7-38.9	28.1-39	38.1-46.1	
LDL						
Mean±SD	122.90±18.84	116.98±43.32	144.72±21.08	148.98±34.17	81.74±18.46	<0.001 O ***
Range	104.54-165.78	43.8-180.38	115.3-180.02	64.1-193.8	54.3-112.3	

n: number of cases., O: one way ANOVA., SD: standard deviation., ***: significant at p = 0.001

Table 6: Comparison of Doppler ultrasound findings among study groups

Characteristic	Choline inositol & metformin n = 30	Metformin Group n = 30	Choline inositol Group n = 30	Newly PCOS n = 30	Normal Control n = 30	p
Follicle volume (mm)						
Mean±SD	17.00±3.91	10.40±2.14	13.30±4.61	6.90±1.67	20.80±0.41	<0.001 O ***
Range	8-21	7-15	7-21	4-9	20-21	

n: number of cases; O: one way ANOVA; SD: standard deviation; ***: significant at p = 0.001

Table 7: Comparison of GLP-1 serum level among study groups

Characteristic	Choline inositol & metformin n = 30	Metformin Group n = 30	Choline inositol Group n = 30	Newly PCOS n = 30	Normal Control n = 30	p
GLP-1 (pmol/L)						
Mean±SD	23.77±11.74	16.36±10.58	22.44±11.86	19.80±10.41	49.42±28.07	<0.001 O ***
Range	6.56-48.56	3.33-31.21	2.78-38.96	6.37-35.98	23.65-95.46	

n: number of cases., O: one way ANOVA., SD: standard deviation., ***: significant at p = 0.001

There was a significant difference in mean FSH (p = 0.001) and the order of groups based on LSD test was as follows: choline inositol + metformin followed by metformin and newly diagnosed groups then by choline inositol and lastly by control.

There was a significant difference in mean LH (p< 0.001) and the order of groups based on LSD test was as follows: metformin and newly diagnosed groups followed by choline inositol and choline inositol + metformin groups and lastly by control.

Rapprochement of Serum Lipid Profile Between Study Groups: Rapprochement of serum lipid profile between study groups is shown in (Table 5). There was significant difference in mean cholesterol (p<0.001) and the order of groups based on LSD test was as follows: newly diagnosed group followed by choline inositol group then by choline inositol + metformin group, then by metformin group and lastly by group of control.

There was magnificent disparity in mean triglyceride (p<0.001) and the order of groups based on LSD test was as follows: newly diagnosed group followed by metformin group, choline inositol group and choline inositol + metformin group and lastly by control group.

There was magnificent disparity in mean HDL (p< 0.001) and the order of groups based on LSD test was as follows: choline inositol + metformin group and control group followed by the rest of groups.

There was magnificent disparity in mean LDL (p< 0.001) and the order of groups based on LSD test was as follows: newly diagnosed group and choline inositol group followed by metformin group and choline inositol + metformin group and lastly by control group.

Comparison of Doppler Ultrasound Findings Between the Group of the Study: Rapprochement of Doppler ultrasound findings between the group of the study is shown in (Table 6). There was magnificent disparity in mean follicle volume (p<0.001) and the order of groups based on LSD test was as follows: control group followed by choline inositol + metformin group then by choline inositol group then by metformin and lastly by newly diagnosed group.

Comparison of GLP-1 Serum Level Among Study Groups: Rapprochement of GLP-1 serum level between the groups of the study is illustrating in (Table 7). There was magnificent disparity in mean serum GLP-1 (p< 0.001) and the order of groups based on LSD test was as follows: control group followed by choline inositol + metformin and choline inositol groups then by newly diagnosed group and lastly by metformin group.

A several of treatment options are nowadays available to treat polycystic ovary syndrome (PCOS). For correct management PCOS in all patients, lifestyle take into consideration. These antiandrogenic progestin should be chosen, in hirsutism, alopecia and acne. To control insulin resistance, body weight and metabolic abnormalities, metformin should be used in overweight/obese adult PCOS women. Metformin may also help you to decrease weight[16].

The mean BMI of new diagnosis control group (G4) is 29.40±5.29 kg/m2, Which explains the fact that PCOS is defined as being overweight. The pathophysiology of an ovulation in PCOS is closely related to obesity. Weight gain and visceral obesity boost insulin resistance and compensatory hyperinsulinemia[17]. Some review research have found that metformin might make clear reduction in the weight's value through appetite regulatory pathways in the brain, which leading to make clear decrease in food uptake, Which means the metformin shows clear benefits on BMI and metabolic outcomes compared with placebo[18]. Gehan Elsawy and *et al.* in 2014 concluded that choline supplementation could hastily reduce body mass without any side influence on biochemical grade or static strength. stated in a clinical study that enhancement in anthropometric parameters when metformin and myoinositol were prescribed in women with PCOS were seen along with better diet. In our study, there are important differences in BMI in groups and we also noticed that BMI is lower in (G3) group and higher in (G4) group when compared with control healthy group (G5) and other groups.

As well as, in our study we included there was no significant difference in mean age (p = 0.255). The means of age were: 27.20±6.28 years, 26.10±4.03 years, 27.10 ±4.07 years, 26.00±6.64 years and 27.67±4.73 years, respectively and the range of age was between 18 and less than 40 years[34].

Inositol and metformin combination was tested against metformin alone by Agrawal *et al*[19]. The findings revealed that the addition of inositol improved menstrual cycles significantly in the former group[19]. This is consistent with our study where we observed that rate of irregular cycle and rate of amenorrhea were improvement in choline inositol and metformin (G3) group when compared with other groups.

Based on our study we found, large follicles volume were significantly higher in choline inositol and metformin group, but small follicles volume were significantly in newly diagnosed (PCOS) Group when

compared with healthy group (G5) and other group[20]. Reported, that their study group (myo-inositol and metformin combination) presented a higher number of large-size follicles (size≥18.0 mm) but small follicles (<14 mm) were higher in metformin alone group that was almost similar to this study[20]. Another study by Xiaoshu Zhan *et al* observed enhanced in follicular development and ovulation when PCOS patients had been taken choline[21].Our data is consistent with previous studies.

In addition, the distribution of LH was significantly different between the groups (p< 0.001) and the order of groups based on LSD test was as follows: metformin and newly diagnosed groups followed by choline inositol and choline inositol + metformin groups and lastly by control healthy group. This suggests that choline inositol and metformin (G3) group and choline inositol (G2) group have lower LH level when compared with G1-G4-G5,at same time mean of metformin alone and newly diagnosed group have a weaker effect on lowering LH compared to G3-G2-G5 groups. Which proves that our study is consistent with[22], that found that Myoinositol with metformin in combination has been shown magnificent procession in the clinical profile with reduction in individual in the dosage of drug in the cases with PCOS[22]. We also note that our study contradicts what the researcher Nabi S et al in their study that suggested the results for LH were not significant; however, the LH/FSH ratio fall was more marked in the metformin group than in the myo- inositol group[23].

In the cases of PCOS, modification in the GnRH pulse frequency leads to increase the serum LH, but normal or low serum FSH. In present study, we also noticed that serum FSH level in new diagnosis group was 5.04±2.06 while FSH in G1-G2-G3 was 5.31±2.33, 4.62 ±2.05, 6.55±3.97 respectively. A significant improvement in FSH level was observed in G2-G3 when compared with new diagnosis (G4) and metformin (G1) group and also G3 group have higher FSH level when compared with other groups. This is in agreement with the findings of the study by[22]. While Martino M. found in their study that choline and inositol lowered LH but had no effect on FSH, this contradicts what was found in our study[12].

In our study, the new diagnosis group showed an abnormal lipid profile in form of decreased HDLc and increased total cholesterol, triglyceride and LDLc ,while the treatment groups Showed improvement in lipid profile. This is consistent with a study by Neha Mishra et al.,which included that groups treatment with berberine hydrochloride, metformin hydrochloride and myoinositol showed a magnificent reduce in total cholesterol's value, serum triglyceride, serum LDL and an increase in serum HDL (p = 0.0001)[26]. The cross-sectional study design by Mohammad Sadegh P. *et al.*, have proven that dietary choline and betaine intakes in obese individuals were related with low levels of pressure of blood and low density lipoprotein (LDL) concentrations[27]. Another study by Iñaki Lete *et al*. Found that inositol is necessary for nerve cell health and lipid metabolism as, together with choline[28]. In our study There was significant difference in mean cholesterol (p<0.001) and the order of groups based on LSD test was as follows: G4-G2-G3 and G1 then G5,which mean that G1 group had more reduction in cholesterol when compared with G2-G3. Also in our study there were significant difference in mean of triglyceride (p<0.001) and LDL (p<0.001), we observed there was no significant difference in mean triglyceride among choline inositol+ metformin, metformin and choline inositol groups. While when compared LDL level among group ,we also noticed that there was no significant between metformin and choline inositol groups but LDL more reduction in G1 and G3 when copared with other groups. In addition to the previous conclusions, we also concluded that adding choline inositol to metformin leads to an increase in HDL level when compared with others groups.

In cretins are peptide hormones, secreted from the gut, responsible for the augmentation of insulin secretion after oral glucose intake[30]. Glucagon-like peptide 1 (GLP-1) is the most important of these. Glucagon-like peptide-1(GLP-1) is L cell-derived peptide in intestinal endocrine . The receptors of GLP-1 are found in islet beta-cells, brain, cardiovascular system, and lung[29]. GLP-1 led to decrease the blood glucose levels through hyperglycemia by inducing the insulin secretion and lowering glucose-dependent glucagon secretion[31]. Few previous studies have addressed the incertain effect in women with PCOS and comparatively few studies have indicated reduced, normal, or increased GLP-1 levels in patients with PCOS[32]. The aimed of this study was to measure GLP1 hormone secretion in female with PCOS and healthy women and to find out the influence of metformin, choline inositol treatment on GLP1 level. Pernille Fog Svendsena *et al*., stated in their study that Metformin increased GIP and GLP-1in lean females with PCOS (P b .05) and a analogues tendency was founded in the obese women (P = .07)[33].

We note that the conclusions of our study are in line with previous studies, as the secretion of the GLP1 hormone was alteration in female with PCOS in a comparison with healthy group. Also there was significant difference in mean serum of GLP-1 between G1-G2-G4 but there was no significant between G1 and

G3 group. Through the difference in mean serum of GLP-1 between G1-G2-G4, we concluded that taking choline inositol with metformin together or choline inositol alone lead to a greater increase in GLP1 level than taking metformin alone. While the search of effect of choline inositol related to GLP1 level in PCOS patients is not found explicitly, therefore, it requires further studies in the future.

CONCLUSION

In our study, we could conclude that the administration of Choline inositol and metformin together causes an increase in FSH, HDL level, GLP1 level and improve amenorrhea, irregularity of cycle and follicle volume after three months as compared with alone Choline inositol administration or alone metformin administration and control group. This combination above are also efficient in reducing BMI, LH level, triglyceride level, LDL level compared to healthy individuals.

REFERENCES

1. Azziz, R., E. Carmina, Z. Chen, A. Dunaif and J.S.E. Laven *et al.,* 2016. Polycystic ovary syndrome. Nat. Rev. Dis. Primers, Vol. 2 .10.1038/nrdp.2016.57.
2. Teede, H.J., M.L. Misso, M.F. Costello, A. Dokras and J. Laven *et al.,* 2018. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. Fertil. Sterility, 110: 364-379.
3. Kshetrimayum, C., A. Sharma, V.V. Mishra and S. Kumar, 2019. Polycystic ovarian syndrome: Environmental/occupational, lifestyle factors: An overview. J. Turk. German Gynecol. Assoc., 20: 255-263.
4. Pasquali, R., 2018. Contemporary approaches to the management of polycystic ovary syndrome. Ther. Adv. Endocrinol. Metab., 9: 123-134.
5. Yuan, M., W. Li, Y. Zhu, B. Yu and J. Wu, 2020. Asprosin: A novel player in metabolic diseases. Front. Endocrinol., Vol. 11 .10.3389/fendo.2020.00064.
6. Ersahin, A.A. and E. Çaliskan, 2018. Clomiphene citrate changes metabolite content of follicular fluid of PCOS women. Eur. Rev. Med. Pharmacol. Sci., 22: 4359-4362.
7. Soldat-Stankovic, V., S. Popovic-Pejicic, S. Stankovic, A. Prtina and G. Maleševic *et al.,* 2021. The effect of metformin and myoinositol on metabolic outcomes in women with polycystic ovary syndrome: Role of body mass and adiponectin in a randomized controlled trial. J. Endocrinol. Invest., 45: 583-595.
8. Advani, K., M. Batra, S. Tajpuriya, R. Gupta and A. Saraswat *et al.,* 2019. Efficacy of combination therapy of inositols, antioxidants and vitamins in obese and non-obese women with polycystic ovary syndrome: An observational study. J. Obstet. Gynaecol., 40: 96-101.
9. Tabrizi, R., V. Ostadmohammadi, K.B. Lankarani, P. Peymani, M. Akbari, F. Kolahdooz and Z. Asemi, 2018. The effects of inositol supplementation on lipid profiles among patients with metabolic diseases: A systematic review and meta-analysis of randomized controlled trials. Lipids Health Dis., Vol. 17 .10.1186/s12944-018-0779-4.
10. Zacchè, M.M., L. Caputo, S. Filippis, G. Zacchè, M. Dindelli and A. Ferrari, 2009. Efficacy of myo-inositol in the treatment of cutaneous disorders in young women with polycystic ovary syndrome. Gynecol. Endocrinol., 25: 508-513.
11. Escobar-Morreale, H.F., 2018. Polycystic ovary syndrome: Definition, aetiology, diagnosis and treatment. Nat. Rev. Endocrinol., 14: 270-284.
12. Diamanti-Kandarakis, E. and A. Dunaif, 2012. Insulin resistance and the polycystic ovary syndrome revisited: An update on mechanisms and implications. Endocr. Rev., 33: 981-1030.
13. Melin, J., M. Forslund, S. Alesi, T. Piltonen and D. Romualdi *et al.*, 2023. The impact of metformin with or without lifestyle modification versus placebo on polycystic ovary syndrome: A systematic review and meta-analysis of randomized controlled trials. Eur. J. Endocrinol., 189: 38-64.
14. Nehra, J., J. Kaushal, S.R. Singhal and V.S. Ghalaut, 2017. Comparision of myo-inositol versus metformin on anthropometric parameters in polycystic ovarian syndrome in women. Int. J. Pharm. Pharm. Sci., Vol. 9 .10.22159/ijpps.2017v9i4.16359.
15. Elsawy, G., O. Abdelrahman and A. Hamza, 2014. Effect of choline supplementation on rapid weight loss and biochemical variables among female taekwondo and judo athletes. J. Hum. Kinet., 40: 77-82.
16. Agrawal, A., R. Mahey, G. Kachhawa, R. Khadgawat, P. Vanamail and A. Kriplani, 2019. Comparison of metformin plus myoinositol vs metformin alone in pcos women undergoing ovulation induction cycles: Randomized controlled trial. Gynecol. Endocrinol., 35: 511-514.
17. Nagaria, T., A. Mohapatra and J. Jaiswal, 2019. Effect of myoinositol and metformin in combination on clinical and hormonal profile in patients of polycystic ovarian syndrome. Int. J. Reprod. Contracep. Obstet. Gynecol., 8: 702-709.
18. Mishra, N., R. Verma and P. Jadaun, 2022. Study on the effect of berberine, myoinositol and metformin in women with polycystic ovary syndrome: A

prospective randomised study. Cureus, Vol. 14 .10.7759/cureus.21781.
19. Abbasi, M.S.P., A.Z. Tousi, Y. Yazdani, S. Vahdat and F. Gharebakhshi *et al.,* 2023. Dietary choline and betaine intake, cardio-metabolic risk factors and prevalence of metabolic syndrome among overweight and obese adults. BMC Endocr. Disord., Vol. 23 .10.1186/s12902-023-01323-4.
20. Pundir, C., R. Deswal, V. Narwal and A. Dang, 2020. The prevalence of polycystic ovary syndrome: A brief systematic review. J. Hum. Reprod. Sci., 13: 261-271.
21. Daniel WW 1998. Biostatistics: A Foundation for Analysis in the Health Sciences. 7th Edn., Wiley, Hoboken, New Jersey, USA., ISBN-22: ISBN-13 978-0471163862, Pages: 928.
22. Deore, D.N., 2012. A cross sectional study on the relationship between the body mass index (BMI) and the audiovisual reaction time (ART). J. Clin. Diagn. Res., 6: 1466-1468.
23. Baggio, L.L. and D.J. Drucker, 2007. Biology of incretins: Glp-1 and gip. Gastroenterology, 132: 2131-2157.
24. Jensterle, M., N.A. Kravos, M. Pfeifer, T. Kocjan and A. Janez, 2015. A 12-week treatment with the long-acting glucagon-like peptide 1 receptor agonist liraglutide leads to significant weight loss in a subset of obese women with newly diagnosed polycystic ovary syndrome. Hormones, 14: 81-90.
25. Muñoz, J.S.G., D.J. Rodríguez and J.J.H. Morante, 2014. Diurnal rhythms of plasma GLP-1 levels in normal and overweight/obese subjects: Lack of effect of weight loss. J. Physiol. Biochem., 71: 17-28.
26. Ferjan, S., M. Jensterle, T. Oblak, I.P. Zitnik and J. Marc *et al.,* 2019. An impaired glucagon-like peptide-1 response is associated with prediabetes in polycystic ovary syndrome with obesity. J. Int. Med. Res., 47: 4691-4700.
27. Svendsen, P.F., L. Nilas, S. Madsbad and J.J. Holst, 2009. Incretin hormone secretion in women with polycystic ovary syndrome: Roles of obesity, insulin sensitivity and treatment with metformin. Metabolism, 58: 586-593.
28. Al-Koofee, D.A.F., J.M. Ismail and A.A. Algenabi, 2019. Lipid profile survey in an adults in an-Najaf/Iraq: A cross-sectional study. J. Phys.: Conf. Ser., Vol. 1294 .10.1088/1742-6596/1294/6/062055.
29. Rani, C., F. Deeba, S. Ishrat, J. Banu and S. Jahan *et al.,* 2022. Effects of metformin plus myo-inositol compared to metformin alone as pre-treatment of ovulation induction in polycystic ovary syndrome with insulin resistance. Int. J. Reprod., Contraception, Obstet. Gynecol., 11: 3005-3011.
30. Zhan, X., L. Fletcher, S. Dingle, E. Baracuhy, B. Wang, L.A. Huber and J. Li, 2021. Choline supplementation influences ovarian follicular development. Front. Biosci. Landmark, 26: 1525-1536.
31. Nabi, S. and R. Guleria, 2018. Comparison of myoinositol and metformin in women with polycystic ovarian syndrome. Indian J. Clin. Practice, 29: 458-463.