The effect of vitamin D, zinc and metformin on poly cystic ovarian syndrome

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Abstract

Background: The polycystic ovary syndrome (PCOS) is the most common endocrine disorder found in women of reproductive age. **Aim of the Study:** The aim of this study is to clarify the effect of zinc, vitamin D, and metformin on poly cystic ovarian syndrome patients.**Patients and methods:** The study was carried out in Tikrit city from 1st of October 2021 to 1st of May 2022 included total of 90 sub-fertile and fertile PCOS women aged 24-35 years old who attend to the department of obstetrics and gynecology at Salah Al-Din General Hospital and private clinics. The PCOS patients were enrolled in a randomized clinical trial and divided into three equal groups. Group A (n:30) received 50 mg of zinc and 400 IU of vitamin D per day, orally. Group B (n:30) received the same as Group A, plus 1,500 mg/day of metformin orally . Group C (n:30) received 1,500 mg/day of metformin orally. Blood sample was taken from each women in the groups in the first visit (2–3 days of menses) before administration of treatment for determination of LH, FSH, serum zinc and vitamin D and base line pelvic songraphy was performed on 2nd and 3rd day from menstrual cycle to asses follicular and ovarian size. Pelvic sonography was performed from day 2 to day 3 of menstruation to asses ovarian and Follicular size. Follicular growth was categorized in to three groups according to the size of follicle using trans pelvic sonography at midcycle. **Results:** The study showed that the administration of Zinc+Vit D+ Metformin have a significant role in regularity of menses as 58% of PCOS women within group B (who received Zinc+Vit D+ Metformin) who were with irregular menstrual cycle became with regular menstrual cycle. The growth of follicles was significantly higher in Group B after treatment and pregnancy occurred in 13% of Group B who received Zinc+ Vit D+ Metformin.**Conclusion:** The study concluded that the administration of Zinc+VitD+Metforminhave beneficial effect in regularity of menstrual cycle decrease in BMI, LH, progesterone and enhance follicular response and increasing pregnancy rate.

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Introduction

The polycystic ovary syndrome (PCOS) is the most common endocrine disorder found in women of reproductive age. Evidences showed that the prevalence of PCOS ranges anywhere from 15 to 20 percent across various countries[(1)]. Despite its prevalence, the exact cause of PCOS remains uncertain. It's a heterogeneous collection of signs and symptoms that form a spectrum of disorders, with mild presentation in some but severe disturbance of reproductive, endocrine and metabolic function in others. The pathophysiology of PCOS appears to be multifactorial and polygenic. The definition of the syndrome has been much debated [(2)]. PCOS runs in families and affect approximately 50% of first degree relatives suggesting a dominant mode of inheritance, commonly first degree male relatives appear more likely to have elevated circulating dehydroepiandrosterone sulfate (DHEAS) levels, early balding, insulin

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resistance and other metabolic alterations typical for PCOS [3] . Studies that focus on pregnancy as an outcome may place a greater emphasis on anovulation as the identifying symptom, rather than the presence of polycystic ovaries or clinical hyperandrogenism. This may be the case because anovulation is easier to detect than the other two symptoms. Women who have hyperandrogenism and have regular menstrual cycles and/or polycystic ovaries appear to be at a significantly lower risk of insulin resistance than women who have chronic hyperandrogenism and have irregular menstrual cycles [4]. It is, therefore, essential that studies into the metabolic features of PCOS should stratify affected women according to ovulatory function (i.e. chronic oligomenorrhea /amenorrhea versus regular cycles). It has been shown that a proportion of PCOS patients do not demonstrate any overt abnormality in circulating androgens [5]. The optimal management of PCOS is uncertain, and treatment focuses on amelioration of the clinical features. The aim of treatment is to restore ovulatory cycles so that pregnancy can be achieved. Clinical studies have shown that metformin (500 mg Chapter One Introduction 2 three times per day or 850 mg twice daily, with meals) administered to women with PCOS increases the frequency of spontaneous ovulation, menstrual regularity[6,7]. However, the long-term use of insulin-sensitizing agents needed to prevent the potential complications of PCOS cannot be recommended because of a lack of evidence regarding their safety and efficacy [8]. Studies done in the past have brought up the possibility that vitamin D also plays a part in reproductive processes. Both the ovary and the testis have been shown to express vitamin D receptors, which provides evidence that vitamin D is active in both of these organs [9] . Zinc (Zn) is an essential trace element that is fundamental for many cellular functions. Zn is involved in many metabolic processes, including carbohydrate, lipid, protein and nucleic acid synthesis and degradation. Recently, zinc administration is proposed for improving clinical and biochemical features of PCOS patients. Zinc is involved as a basic element for many vital functions including fertility and reproduction [10,11].

Patients and Methods

The study was carried out in Tikrit city from 1st of October 2021 to 1st of May 2022 at Salah Al-Din General Hospital and private clinics. Our study included total of 90 sub-fertile and fertile PCOS women aged 24-35 years old who attend to the department of obstetrics and gynecology at Salah Al-Din General Hospital and private clinics. An interview was carried out with these patients using questionnaire form including their demographic characteristics, age, weight, height, amenorrhea, oligomenorrhea, drug history and family history.

Patients were divided into three groups , each containing 30 women , using random number table . Group A received 50 mg of zinc and 400 IU of vitamin D per day, orally , Group B received the same as Group A, plus 1,500 mg/day of metformin orally and Group C received 1,500 mg/day of metformin orally . The metformin dose was increased stepwise (starting with 500 mg once daily for the 1st week and 500 mg twice daily in the 2nd week, followed by 500 mg 3 times daily from the 3rd week

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onward; marc ,France) , The dose of zinc (50mg/daily ; natrol , USA) and vitamin D (400 IU/daily; natrol , USA) remained constant throughout the study period.

Before giving treatment (Zinc , vitamin D and metformin) diagnostic endocrine tests included serum levels of follicle-stimulating hormone (FSH) , luteinizing hormone (LH) and LH/FSH ratio (2nd and 3rd day from menstrual cycle) . Serum levels of Zinc and vitamin D also measured.

Three ml of blood sample was taken by vein puncture from each women in the groups in the first visit (2–3 days of menses) before administration of zinc, vitamin D and metformin. Blood samples were placed into sterile test tubes, after blood clotting, centrifuged at 3000 rpm for 15 minutes then clot removed and remain re-centrifuged at 3000 for 10 minutes and the obtained serum were aspirated using mechanical micropipette and transferred into clean test tubes which labelled and stored in deep freeze at -20 c for determination of LH, FSH,LH/FSH ratio serum zinc and vitamin D.

Base line pelvic sonography was performed on 2nd and 3rd day from menstrual cycle to asses follicular and ovarian size . In order to determine follicular growth, pelvic sonography was performed from day 9 to day 12 of menstrual cycle.

Results

1.Demographic characteristicsof thestudied groups

The studyshowed nosignificant differenceamong thethree studiedgroups regarding themeanlevelsofageand BMI(P>0.05)andmoststudiedwomenhave highBMI levels,Table 1.

Table 1: Demographic characteristicsof thestudied groups

Parameters	GroupA	Group B	GroupC	P. value
BMI(Kg/m^2)	27.18±2.23	27.42±2.44	27.38±2.57	>0.05
Age (year)	27.86±5.14	29.97±6.24	28.47±5.49	>0.05

Group A:received zinc+vitGroup B:Zinc+VitD+Met Group C: Metformin only

2.Clinicalcharacteristicsof thestudied groups

The studyshowed no significant difference amongthe three studiedgroups regarding theclinicalcharacteristics (P>0.05). Thestudyalso showedthatmostof women withPCOS have,hirsutism and Amenorrhea,Table 2

Table 2: Clinicalcharacteristicsof the studied groups

Variables		GroupA		GroupB		GroupC		P. value
		No.	%	No.	%	No.	%	
Acne	Present	11	37	10	33	8	27	>0.05
	Absent	19	63	20	67	22	73	
	Total	30	100	30	100	30	100	

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Hirsutism	Present	21	70	22	73	20	67	>0.05
	Absent	9	30	8	27	10	33	
	Total	30	100	30	100	30	100	
Menstrualcycle	Yes	3	10	4	13.33	3	10	>0.05
	No	27	90	26	86.67	27	90	
	Total	30	30	100	30	100	30	
Oligomenorrhea and Amenorrhea	Oligomenorrhea	10	37.04	8	30.77	12	44.44	>0.05
	Amenorrhea	17	62.96	18	69.23	15	55.55	
	Total	27	100	26	100	27	100	
Familyhistory	Present	13	43	11	37	9	30	>0.05
	Absent	17	57	19	10	21	70	
	Total	30	100	30	100	30	100	

3.Levelsof LH,FSH,LH/FSH ratioamong the three studiedgroups

The studyshowednosignificantdifference amongthe threestudiedgroups regardingthe meanlevelsof LH,FSH,LH/FSHratio(P>0.05),Table3.

Parameters	Group A	Group B	Group C	P. value
No.	30	30	30	
LH (mIU/mL)	9.92±4.24	10.51±4.31	9.01±4.54	>0.05
FSH (mIU/mL)	5.91±1.02	4.99±1.69	5.75 ±0.75	>0.05
LH/FSH ratio	1.74±0.78	1.95±0.74	2.01±0.18	>0.05

Table 3: LevelsofLH,FSH,LH/FSHratioamongthe three studiedgroups

4.Levelsof vitamin D andserumzincamong the three studiedgroups

The studyshowednosignificantdifference amongthe threestudiedgroupsin the1st visitregarding themean levelsof vitaminDandzinc and thethreegroupshave vitaminDdeficiencyandlower limitof serum zinc (P>0.05),Table4.

Table 4: LevelsofvitaminDand serumzinc amongthe three studiedgroups

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Parameters	GroupA	Group B	GroupC	P. value
No.	30	30	30	
Vitamin D (ng/ml)	11.38±3.91	14.01±4.28	13.07±4.59	>0.05
Zinc (µg/dL)	53.81±16.42	56.87±18.01	54.92±20.83	>0.05

Normalrange:Zinc50-103µg/dLforfemale, Vit.D:30-100normal,10-30dificient

5. Meanof ovarian volume ,follicularsizesand 21st dayProgesterone of the three studied groups.

The studyshowed nosignificant differenceamong thethree studiedgroups regardingthemeanof ovarianvolume, follicularsizesand21st dayProgesterone in the 1st visit before treatment(P>0.05),Table 5

Table 5: Meanofovarian volume ,follicular sizesand 21st dayProgesterone of the three studied groups

Parameters	GroupA	Group B	GroupC	P. value
Follicular size (mm)	9.61±0.55	8.63±0.34	9.62±0.52	>0.05
Sizeof ovaries (cm^3)	10.05±0.51	9.12±0.73	10.09±0.49	>0.05
21st day Progesterone (ng/ml)	7.88±1.56	8.86±1.82	7.97±1.72	>0.05

6. Mensesregularityafter treatmentof the studiedgroups

The study showed that the administration of Zinc+Vit D+ Metformin have a significantrole inregularity ofmenses as58% of PCOS womenwithin groupB (whoreceivedZinc+VitD+ Metformin)whowere withirregularmenstrualcycle becamewithregularmenstrualcycle,comparedwith30%of group A(whoreceived zinc+vitD)26%ofGroupC(whoreceivedonlyMetformin),(P:0.033),Table6

Table 6: Mensesregularityafter treatment of thestudiedgroups

Mensural cycle	GroupA		Group B		GroupC	
	No.	%	No.	%	No.	%
Regular	8	30	15	58	7	26
Irregular	19	70	11	42	20	74
Total	27	100	26	100	27	100

Group A:received zinc+vitD Group B:Zinc+VitD+ Met Group C: Metforminonly

7.Changesin BMI after treatmentofthe studiedgroups

The study showed that theadministration of Zinc+VitD+Metformin havean enhance roleinreduction ofBMI after treatmentas GroupBhave decreasedBMI level(P:0.003) ascompared with group A and groupB.Table 7.

Table 7: Changes in BMI after treatment of thestudiedgroups

BMI (Kg/m²)	Group A	Group B	Group C	P. value
Before treatment	27.18±2.23	27.42±2.44	27.38±2.57	A & B: >0.05 A & C: >0.05 B & C: >0.05
After treatment	25.14±2.13	24.04±3.34	25.45±3.45	A & B: 0.038 A & C: >0.05 B & C: >0.43
P. value	0.042	0.003	0.004	

8.Changes in hormonal levels after treatmentof thestudied groups

The studyshowed thatthe receivingof Zinc+VitD+Metformin havean enhanced affect inreduction of LH and LH/FSH ratio while no significant difference in the meanFSH levels ininall studied groupsafter treatment (P>0.05), Table 8.

Table8: Changes in hormonallevels after treatment of the studied groups

Parameters		Group A	Group B	Group C	P. value
LH (mIU/mL)	Before treatment	9.92±4.24	10.51±4.31	9.01±4.54	>0.05
	After treatment	9.49±4.13	8.58±3.68	8.89±4.33	0.21
	P. value	>0.05	0.023	>0.05	
FSH (mIU/mL)	Before treatment	5.91±1.02	4.99±1.69	5.75 ±0.75	>0.05
	After treatment	6.26±1.07	6.25±0.72	6.32±0.77	>0.05
	P. value	>0.05	0.021	>0.05	
LH/FSH ratio	Before treatment	1.74±0.78	1.95±0.74	2.01±0.18	>0.05
	After treatment	1.64±0.73	1.59±0.51	1.99±77	>0.05

9.Changes inovarianvolume,follicular sizesand 21st dayProgesterone after treatment of the studiedgroups

The studyshowed thattheadministration ofZinc+VitD+ Metformingavean improvedaffectinreductionofovarianvolumeandelevationoffollicular size serum andincreasinginlevelsofprogesteroneasthemeanovarianvolumeofwomen within GroupB decreasedsignificantly(8.67±0.46mm) withsignificantincrease in follicular size ($13.98\pm3.68cm^3$) and serumprogesterone level(12.06±0.99ng/ml) afterreceivingZinc+VitD+Metformin(P:0.001)ascomparedwithgroupsAandC ,Table 9.

Table 9: Changes inovarian volume , follicular sizes and 21st day Progesterone after treatment of thestudied groups

Parameters		GroupA	Group B	GroupC	P. value
Follicular size (mm)	Before treatment	9.61±0.55	8.63±0.34	9.62±0.52	>0.05
	After treatment	12.55±0.67	13.98±3.68	12.91±4.33	0.044
	P. value	0.043	0.001	>0.05	
Sizeof ovaries (cm^3)	Before treatment	10.05±0.51	9.12±0.73	10.09±0.49	>0.05
	After treatment	9.32±0.51	8.67±0.46	9.93±0.77	>0.05
	P. value	>0.05	0.043	>0.05	
21st day Progesterone (ng/ml)	Before treatment	7.88±1.56	8.86±1.82	7.97±1.72	>0.05
	After treatment	11.88±1.83	12.06±0.99	11.21±0.74	0.041
	P. value	>0.05	0.001	>0.05	

10. Follicular response after treatmentof the studied groups

AsshowninTable10,thefollicularresponsein2nd visitwassignificantly higher inGroupwhoreceivedBafter 2monthsoftreatmentwithZinc+VitD+ Metformin thaninGroupC(p=0.04)and GroupA(0.45)andthestudyshowedthat ,thefollicularresponseinlastvisitwasalsorelativelyhigherinGroupBafter2 months of treatment.

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Date of visit	Follicular response	Group A		Group B		Group C	
		No.	%	No.	%	No.	%
2nd visit	No response	25	83.33	20	66.67	26	68.67
	Borderline (10-14 mm)	2	6.67	2	6.67	3	10
	Responded >14	3	10	8	26.67	1	3.33
	Total	30	100	30	100	30	100
P. value: (A and C: 0.45) (A and B: 0.24) (B and C: 0.04)							
Last visit	No response	20	66.67	16	53.33	19	63.33
	Borderline (10-14 mm)	4	13.33	4	13.33	7	23.33
	Responded >14	6	20	10	33.33	4	13.33
	Total	30	100	30	100	30	100
P. value: (A and C: 0.22) (A and B: 0.022) (B and C: 0.029)							

Table 10:Follicular responseafter treatmentof the studied groups

11.Pregnancy rate after treatment of the studiedgroups

As shown inTable 11, pregnancy occurred in13%(4of 30) ofGroupBwho received Zinc+ VitD+MetforminwhichwassignificantlyhigherthanGroupC(P: 0.038) and GroupA who have pregnancyrate 7%(2 of 30) (P:0.15).

Table 11: Pregnancyrate after treatment of the studied groups

Pregnancyrate	GroupA		Group B		GroupC	
	No.	%	No.	No.	%	No.
Occurred	2	7	4	13	0	0
Failed	28	93	26	87	30	100
Total	30	100	30	100	30	100
P.value: AandC: 0.15 AandB: 0.38 B andC:0.038						

5. Discussion

PCOS is recognized as one of the most common female endocrine disorders, and is characterized by hyperandrogenic chronic anovulation with infertility, irregular menses, and hirsutism (12) . Our study was to clarify the effect of zinc, vitamin D, and metformin on polycystic ovarian syndrome patients. The study showed no significant difference among the three studied groups

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in the 1st visit regarding the mean levels of vitamin D and zinc and the three groups have vitamin D deficiency and lower limit of serum zinc (P>0.05), Table 4.4.4. Thomson et al (13) found that vitamin D deficiency may exacerbate symptoms of PCOS, which was associated with insulin resistance, ovulatory and menstrual irregularities, lower pregnancy success, hirsutism, hyperandrogenism, obesity and elevated cardiovascular disease risk factors and there is some, but limited, evidence for beneficial effects of vitamin D supplementation on menstrual dysfunction and insulin resistance in women with PCOS. Foroozanfard et al(14) conducted a randomized, study in women with PCOS (n = 52; 18–40 years old) to examine the effect of zinc supplementation on the metabolic profile of women with PCOS. Women were randomly divided into two groups. The treatment group received a supplement of 220 mg zinc sulfate (containing 50 mg elemental zinc) per day for 8 weeks. Compared to other group, Calcaterra et al(15) concluded taht zinc supplementation in women with PCOS resulted in a reduction in fasting plasma glucose (FPG) (−4.3 mg/dL, p = 0.03), serum insulin (−3.0 µIU/mL, p = 0.01), HOMA-IR (−0.8, p = 0.006), , serum triglycerides (−15.6 mg/dL, p = 0.002), and very-low-density-lipoprotein (VLDL) cholesterol (−3.2 mg/dL, p = 0.002) and an increase in the quantitative insulin sensitivity check index. But this didn't agree with our study that zinc effect on follicular growth and regularity of menstrual cycle.

Conclusions.

1. The study concluded that the administration of Zinc+Vit D+ Metformin have beneficial effect in regularity of menstrual cycle.
2. The study concluded the effects of metformin and Zinc+Vit D in reduction of BMI.
3. The study demonstrated that the receiving of Zinc+VitD+Metformin have an enhanced affect in reduction of LH and LH/FSH ratio but FSH levels slightly increase.
4. The administration of metformin and Zinc+Vit D have beneficial effects on follicular growth and increasing in serum levels of day 21 progesterone.
5. The study concluded that pregnancy rate increase with administration of metformin and Zinc+Vit D.

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