Combined chromium with letrozole versus letrozole only in induction of ovulation in patients with polycystic ovary syndrome: A Randomized controlled trial

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Abstract

Background: polycystic ovary syndrome (PCO) is the most common cause of anovular infertility. Many researches were done to improve ovulation in this syndrome. Many demographic, hormonal and biochemical changes are associated with this syndrome. This incluces obesity, high insulin resistance and high LH/FSH ratio. Chromium picolinate by itself helps in reduction of body mass index and reduces insulin resistance. Aim of work: we aim to check if the use of chromium picolinate can improve ovulation in polycystic ovary symdrome as it improves most of associateddemographic and hormonal factors associated with PCO. Patients and method: 90 infertile patients suffering from PCO were divided into two groups. Group A (45 patients) were given aromataze enzyme inhibitor (Letrozol) and Group B (45 patients) were given chromium picolinate in addition to Letrozol. Results: significant improvement of ovulation and pregnancy percentages in group B compared to group A. additionally all demographic and hormonal changes associated with PCO were improved significantly in most of them. Conclusion: Adding chromium picolinate potentiates the therapeutic effect of letrozol on pituitary hormones (FSH and LH), reduction of body mass index, improvement of ovulation, normalization of Estrogen level, improvement of endometrial thickness related to ovulation, improvement of progesterone production after good ovulation, reduced fasting insulin and reduction of androgen levels.

Keyword: *chromium picolinate, Letrozol, PCO, polycystic ovary, anovulation.*

INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common form of WHO type II anovulatory infertility and is associated with symptoms and signs of hyperandrogenism, its prevalence ranges from (11.9 ± 2.4%) to (17.8 ± 2.8) according to Rotterdam diagnostic criteria (1Overbeek et al ., 2009 ;2 March et al ., 2010)

The initial step in the biosynthesis of all steroid hormones is the conversion of cholesterol to pregnenolone, by a two-stage process involving cholesterol side chain cleavage enzyme and the acute steroidogenic

regulatory protein, pregnenolone is then converted to dehydroepiandrosterone (DHEA) by a two-step process along the Δ5-steroid pathway, with the conversion being catalysed by cytochrome P450c17a.

Chromium picolinate has been shown in many studies to having efficacy on reduction of BMI, and improving insulin sensitivity and decreasing hyperinsulinemia, improving menstrual regularity and symptoms and signs of hyperandogenism, giving better chances as adjuvant therapy in increasing fertility outcome (3 Lydic et al ., 2006)

Patients and Methods

This prospective study was done on ninety of infertile female patients who already were diagnosed with Polycystic ovary syndrome in outpatient clinic of kasr al Ainy university hospital and they failed to conceive for more than one year despite of regular coitus without any methods of contraception despite of absence of male factor.

Inclusion Criteria included Female patients aged from 18 to 40 years, Normal uterine cavity with patent both tubes by hysterosalpinography, Anovulatory cycle diagnosed by ultrasound folliculometry day11 and day13 from cycle and Normal semen analysis of husband. Patients were diagnosed according to Rotterdam criteria of PCOS. Diagnosis is confirmed by 2 of 3 criteria after exclusion of the other etiologies, Oligo or anovulation, Biochemical and /or clinical signs of hyperandrogenism. Biochemical: Total testosterone >70 ng/dl. Androstenedione>245 ng/dl DHEA –S >248 ng/dl, clinical : Acne, Hirsutism ,acanthosis nigrans, polycystic Ovaries (>12 follicles from 2mm to 9mm in each ovaries or ovarian volume more than 10cc) and with or without glucose tolerance test .

Exclusion Criteria included: Female patients aged more than 40 years . Male factor of Infertility . Congenital or acquired factor of infertility asTubal factor. Uterine factor as asherman syndrome, septate uterus .., etc. Severe hormonal disturbance as Hypothyroidism HypopituitarismProlactinoma.. ,etc

Methods:For all women, a Verbal consent was taken after explanation of nature of study including duration of drug exposure and possible side effects .A Complete history and clinical examination were done to adequately include and exclude cases according to criteria of the study. Pelvic ultrasound was performed. Hysterosalpinography was performed at proper time before induction to exclude any tubal blockage.

All patients had been investigated for Semen analysis Serum FSH & LH and estradiol (E2). Serum prolactin. Thyroid function test (TSH). Fasting blood insulin . Fasting blood glucose & 2hrs postprandial blood glucose. Total and Free Testosterone. DHEA-S and Serum progesterone at day 21 of cycle.

After enrollment, 90 patients were randomly divided into equally distributed two groups as ratio of 1:1 by using identical sealed envelopes technique into: (n=45) for each group. Group A : (n=45): Pateints received letrozole 2.5 mg tabs once from day 2 of cycle for 5 days. Group B : (n =45): receiving only chromium picolinate 200 mcgm once daily at first for two cycles allover 2 months daily then they received letrozole in addition as combined therapy .

All cases were followed up for 6 cycles by transvaginal ultrasound folliculometry to document ovulation. Folliculometry was done on day 11, 13 of the cycle to detect number of growing follicles and size of follicles. Follicles measure more than 18mm were considered mature follicles.Patients with no follicles or less than 15 mm in diameter were considered as non responders. Patients with one or more than one follicle equal or more than 18 mm in

diameter were considered as good responder to induction.

Serum β-HCG was measured if the menses was delayed for one week for diagnosis of pregnancy chemically then followed up by TVS to detect gestational sac at (4 weeks of gestation) and fetal pulsation at (7 weeks of gestation).

Monthly follow up of biochemical and clinical signs of hyperandrogenism and menstrual irregularities, serum insulin and glucose level, LH/FSH Ratio, body mass index of patients to detect effect of chromium supplementation as adjuvant therapy with induction of ovulation to PCOS patients.

Data was analyzed using SPSS version 19 (SPSS Inc., Chicago, IL, USA) software. Normal distribution of the studied variables was evaluated using Kolmogorov-Smirnov test. Mean (standard deviation [SD]) of studied variables in two studied groups were compared using the independent t-test.

For each group, pre and post usage of the treatment was assessed using the paired samples test on SPSS the T-Test gives us through the P-Value whether the difference between "Pre" values and "Post" values is significant or not.

P value is considered significant if the P-value less than or equal to 0.05 and greater than 0.01, and highly significant for P-value less than or equal to 0.01.

Results:

Demographic data and symptoms of the studied patients are presented in table 1and 2. There is no significant difference between the two groups regarding age and duration of infertility.

Table 1: Quantitative demographic and infertility dataof the examined patients

Variable	Group	Min	Max	Mean	SD	P-Value
Age	A	20	36	27.58	4.26	0.346
	B	20	36	26.67	3.86	
Duration of infertility	A	2	9	4.27	1.54	0.674
	B	2	8	4.13	1.65	

Table 2: Qualitative demographic and endocrine symptoms data

Variable	Status	Group	Freq.	Percent %
Family history of diabetes mellitus	Free	A	28	62.2
		B	29	64.4
	Positive	A	17	37.8
		B	16	35.6
Type of infertility	Primary	A	34	75.6
		B	35	77.8
	secondary	A	11	24.4
		B	10	22.2
History of contraception	None	A	40	88.9
		B	40	88.9
	Yes	A	5	11.1
		B	5	11.1
hirsutism	No	A	13	28.9
		B	11	24.4

	Yes	A	32	71.1
		B	34	75.6
Menstrual irregularity	non	A	8	17.8
		B	8	17.8
	Yes	A	37	82.2
		B	37	82.2
oligomenorrhoea	No	A	8	17.8
		B	8	17.8
	Yes	A	37	82.2
		B	37	82.2
Facial acne	No	A	22	48.9
		B	24	53.3
	Yes	A	23	51.1
		B	21	46.7

Patients' hormonal profile on start of the study is presented in table 3. There are no significant differences between the two groups.

Table 3: hormonal profile of the two studied groups.

Variable	Group	Min	Max	Mean	SD	P-Value
Prolactin (ng/ml)	A	4	23	14.62	5.27	0.280
	B	5	22	13.44	4.43	
TSH (µU/ml)	A	1.3	4.4	2.75	0.85	0.669
	B	0.9	4.3	2.83	0.84	

There is a significant reduction of BMI in group B after use of chromium (table 4)

Table 4 metabolic and hormonal changes after use of studied medications in both groups

Comparing items before and after treatment	Mean difference	T-Ratio	P-Value
BMI in group B	0.893	12.289	P<0.001
FSH group A (rise)	-0.2	-2.146	P<0.05
FSH group B (rise)	-0.156	-1.551	0.128
LH group A (drop)	0.889	8.748	P<0.001
LH group B (drop)	3.400	11.509	P<0.001
LH/FSH ratio group A (dropped)	0.288	7.212	P<0.001
LH/FSH Ratio group B (dropped)	0.655	12.054	P<0.001
E2 group A (dropped)	3.669	0.572	0.570
E2 group B dropped	7.387	4.729	P<0.001
Total Testosterone group A (rise)	-0.556	-0.415	0.680
Total Testosterone group B (drop)	11.333	12.893	P<0.001
Free Testosterone group A (rise)	-0.805	-1.043	0.303
Free Testosterone group B (drop)	0.077	5.025	P<0.001
DHEA-S Pre. A - DHEA-S Post A	7.733	4.846	P<0.001
DHEA-S Pre. B - DHEA-S Post B	19.956	12.078	P<0.001
Fasting blood glucose Pre. A - Fasting blood	1.289	1.264	0.213

glucose Post A			
Fasting blood glucose Pre. B - Fasting blood glucose Post B	3.733	3.910	P<0.001
Fasting blood glucose Pre. A - Fasting blood glucose Post A	1.289	1.264	0.213
Fasting blood glucose Pre. B - Fasting blood glucose Post B	3.733	3.910	P<0.001
Fasting blood insulin Pre. A - Fasting blood insulin Post A	0.298	1.116	0.270
Fasting blood insulin Pre. B - Fasting blood insulin Post B	4.671	13.106	P<0.001
Fasting Glucose insulin Ratio Pre. A - Fasting Glucose insulin Ratio Post A	-0.070	-0.421	0.676
Fasting Glucose insulin Ratio Pre. B - Fasting Glucose insulin Ratio Post B	-2.122	-12.752	P<0.001
Day 21 serum progesterone Pre. A - Day 21 serum progesterone Post A	-5.759	-6.051	P<0.001
Day 21 serum progesterone Pre. B - Day 21 serum progesterone Post B	-7.524	-7.285	P<0.001

NB: negative sign means that results increased after the used medication compared to the premedication.

The results showed that BMI was highly significant with high T-Ratio and the decreasing in BMI became more distinguishable, these results indicating good effect on weight reduction .

Letrozole wasn't considered in the study regarding efficacy on BMI reduction, as normally aromatase inhibitors have no effect on body weight reduction

The results showed that FSH increased in group A significantly (P<0.05). Adding chromium (group B) the FSH rise is insignificant. LH levels, LH/FSh ratio decreased signinficantly in both groups.

Estrogen, total testosterone, free testosterone, fasting and postprandialblood glucose, fasting insulin, and fasting insulin /glucose ratio levels decreased in both groups but this was significant only with the use of chromium (group B). Dehydro epiandrosterone acetate decreased significantly in both groups. Progesterone levels increased significantly in both groups

Table 5: Improvements of clinical symptoms are presented in table

group	**Improvement of Clinical Symptoms**	**Number of cases**	**Percentage**
A	Menstrual irregularity –Oligomenorrhea	22	48.89%
	No clinical improvement	20	44.44%
	No clinical symptoms	3	6.67%
total		**45**	**100.00%**
B	Hirsutism	17	37.8%
	Facial acne	12	26.7%
	Menstrual irregularity	26	57.8%

	-Oligomenorrhea		
	No clinical improvement	9	20.00%
	No clinical symptoms	8	17.78%

The results showed that adding chromium picolinate to letrozole achieved the best result, as only 22.22% of the sample remained without dominant follicle. That reveals in the appended graphs. (Table 6)

Table 6: folliculometry in both groups

	U/S Folliculometry day 11 ,day 13	**Number of cases**	**Percentage**
A	No Dominant follicle was observed	16	35.56%
	One Dominant follicle measuring >= 18 mm	23	51.11%
	Two Dominant follicle measuring >= 18 mm	6	13.33%
	Total Number of cases with mature follicle	29	64.44%
total		**45**	**100.00%**
B	No Dominant follicle was observed	10	22.22%
	One Dominant follicle measuring >= 18 mm	26	57.78%
	Two Dominant follicle measuring >= 18 mm	9	20.00%
	Total Number of cases with mature follicle	35	77.78%
Total		**45**	**100.00%**

The results showed that adding chromium picolinate to letrozole achieved the best result, as 77.78% of the sample positive for Ovulation (table 7)

Table 7: Primary outcome (ovulation)

	Primary Outcome	**Number of Cases**	**Percentage**
A	Anovulation	16	35.56%
	Ovulation after one cycle	12	26.67%
	Ovulation after two cycles	9	20.00%

	Ovulation after three cycles	8	17.78%
	Cumulative number and percentage of cases who ovulated	29	64.44%
total		**45**	**100.00%**
B	Anovulation	10	22.22%
	Ovulation after one cycle	14	31.11%
	Ovulation after two cycles	13	28.89%
	Ovulation after three cycles	8	17.78%
	Cumulative number and percentage of cases who ovulated	35	77.78%
total		**45**	**100.00%**

Adding chromium picolinate to letrozole achieved the best result, as 29% of the sample found with Positive pregnancy test (table 8)

Table 8: Secondary outcome successful pregnancy.

Group	**Secondary Outcome**	**Number of cases**	**Percentage**
A	Negative pregnancy test	36	80.00%
	Positive pregnancy test	9	20.00%
total		**45**	**100.00%**
B	Negative pregnancy test	32	71.11%
	Positive pregnancy test	13	28.89%
total		**45**	**100.00%**

Discussion

Polycystic ovary syndrome (PCOS) is the most common form of WHO type II anovulatory infertility and is associated with symptoms and signs of hyperandrogenism, according to Rotterdam diagnostic criteria (1 ,2).letrozole is an aromatase inhibitor with less anti estrogenic side effects on endometrium and cervical mucus with higher mono follicular selectivity growth comparing with clomiphene citrate and other drug inducer so it has better fertility outcome and avoiding hazards of multiple pregnancy (4).

In present study ninety patients (n=90) presented to Kasr Al-Ainy hospital outpatient clinic and diagnosed with PCOS were randomly distributed into two equal groups as ratio 1:1.

Demographic data showed no significant differences between the two studied groups (age, duration of infertility and BMI). Additionally, there was insignifican difference between the two groups regarding thyroid function and prolactin level. BMI was significantly reduced in group B (given chromium) compared to group A. Pittler et al

in 2003(5) reported similar resultsbut this was insignificant in diabetic patients. On the other hand, many studies denied any effect from chromium use on BMI, fat or iron metabolism (6).

Regarding pituitary hormones, FSH levels increased significantly in group A (p-value = 0.037). It also increased in group B (chromium plus letrozol) but this was insignificant (p-value =0.128). it is probably due to higher duration of exposure to patients among group A to letrozole therapy for 6 months whilegroup B patients received chromium picolinate for two months before using letrozole therapy as drug inducer for 4 months only.

More important, patients in both groups showed highly significant reduction of LH level. The patients among group B who received combined therapy had greater effect on LH level decline (t-ratio=11.509), it is mostly that receiving chromium can affect LH level with correlation with reduction of BMI and subsequently reduction of testosterone level later on .

Large Prospective RCTs study by Elizabeth et al in 2011(7) showed that letrozole (5-7.5mg) daily, showed increased ovulatory rate and pregnancy rate as (11%) of cases got pregnant, with increasing effect on FSH with non significant effect on LH, so LH/FSH was increased and decreased effect on serum Estradiol. Badawy et al(8) showed longer exposure to letrozole provides a longer period of elevated FSH levels in which follicular development can occur, which may account for the higher pregnancy rate with the long course regimen .

Regarding androgen level in blood in women with PCOS (patients received chromium in group B resulted in highly significant reduction in total testosterone (p-value= zero) indicating more effect with continuous administration of chromium picolinate) .

Free testosterone level among group A patients insignificantly increased as letrozole had no desirable effect on free testosterone. Patients among group B who continued to receive chromium with letrozole, showed highly significant reduction (p-value =zero).

It should be noted that levels of total or free testosterone are affected by other variables as albumin level, sex hormone binding globulin (SHBG) which is also affected by insulin level and estrogen level.

Dehydroepiandrosterone sulphate (DHEA-S) is significantly decreased in both groups of patients (p-value =zero) but the decrease is more in the chromium group (group B).

Seven randomized controlled trials studies by Siavash Fazelian et al(9) concluded the same results. This agreed also with Sherif Ashoush et al(10), Results indicated that Cr supplementation had a beneficial effect on BMI with effect (p-value = 0.001) ,free testosterone concentration with effect (p-value=.001), reduced fasting insulin (p-value=.001) indicating significant effect, but results showed in contrast to our study that no beneficial effects on reducing total testosterone, DHEA, FSH and LH .

On the other hand, other studies (3,11) concluded that chromium picolinate in women with polycystic ovary syndrome does not improve ovulatory frequency or hormonal profile such as androgen level.

Hyperinsulinemia is the cornerstone of PCOS pathophysiology, hence fasting blood insulin results in present study showed that patients in group A who received letrozole only had insignificant effect (p-value =0.270). On other hand, fasting insulin level and fasting blood glucose significantly reduced in group B (P value=zero). This is indicating effective therapy with continuous administration of chromium picolinate. This is probably due to reduction of body weight with subsequently

reduction of androgen level among patients receiving chromium .

In agreement with present study, other studies (12,13) showed that Chromium picolinate significantly decreased fasting blood sugar (FBS) after 3 months of treatment (p=0.042). In the same way, the serum levels of fasting insulin had significantly decreased leading to an increase in insulin sensitivity as measured by QUICKI index (p=0.014), But in contrast with our study, the study failed to prove efficacy of lowering testosterone level as metformin did .

Other studies concluded that there is no association between chromium and glucose or insulin concentrations for non-diabetics, also they failed to show any improvement in insulin resistance or reduction in insulin or glucose level for participants and inconclusive results for diabetics (14,15)

Patients among group A who were given letrozole had significant Menstrual Irregularities and Oligomenorrhea improvement (49%) and no other improvement concerning Acne or Hirsutism.

Patients in group B with continuous intake of chromium with letrozole showed higher and significant improvement in Menstrual Irregularities and oligomenorrhea (57.8%), Hirsutism (37.8%) ,Acne (26.7%), remain only (20%) without clinical improvement, assuming improvement in using chromium therapy which have lowering effect on serum androgen level, but with little clinical value on androgenic symptoms .

In agreement with our study, Jamilian et al (16) agreed with Nermin Amr et al in improvement of ovulation and positive effect on menstrual regularity that was made by chromium, as Thirty five female patients with PCOS were included, high dose of chromium picolinate (1000 μg) daily was obtained for 6 months, results showed significant improvement of menstrual irregularities and oligomenorrhea (83%)(P-value < .001), Significant reduction in mean ovarian volume (P < .001), total follicular count (P-value < .034), and free testosterone (P< .002) was observed but in contrast to our study showed that there was no significant improvement in acne or hirsutim .

With agreement to our study, Kolodziejczyk et al(17), showed that the presence and degree of hirsutism, alopecia and acne were reduced after 2 months of treatment with chromium. chromium group had a greater pregnancy rate (P=0.08) and decreased alopecia (P=0.22) howerever wasn't achieved its target to be significant.

Also using letrozole only reflects significant improving in menstrual irregularities in PCOS women (18) .

Also in agreement with us Mehri et al(19) showed that chromium had a significant reduction in acne (P=0.04), hirsutism (P=0.002), serum C-Reactive protein (P=0.02), plasma MDA (P<0.001), and a significant rise in TAC concentrations (P<0.001) compared to the placebo group .

In present study ,concerning Follicle maturation follow up by transvaginal ultrasound at Day 11, Day 13 results showed that there was a significant improvement in follicular growth with patients received letrozole only for induction of ovulation to those patients (64%) had Dominant follicles growth measuring (>= 18 mm) .

Among group B patients who received chromium picolinate with added letrozole, the results showed significant improvement in follicle growth and maturation as measuring dominant follicles more than 18mm was (78%) .

On the other hand, number of mono follicular growth was (51.11%) of total cases in group A who received letrozole only but with two follicle growth (13.33%) . In group B, patients

was found to be higher in in mono follicular growth (57.78%) and significant higher two follicular growth (20 %) which means that the addition of chromium picolinate affects positively in growth of more than one follicles with increasing chances of ovulation .

Net result was that the ovulation occurred with letrozole only in group A had significant results (64%), higher results of ovulation with best outcome was achieved when combined therapy was used in group B.

Results also showed that adding chromium picolinate to letrozole gave highly significant results (p-value=zero) regarding serum progesterone level at day 21 of cycle .

In agreement with our study, Legro et al(18) and Atay et al(20), in large prospective study in 750 patients with PCOS comparing efficacy of letrozole versus clomiphene citrate regarding ovulation and menstrual irregularities improvement showed significant results for letrozole vs clomiphene citrate in ovulation (61.7% vs 48.3%) respectively, also there is higher fertilization rate for letrozole as women who got live births (27.5 vs 19.1) respectively .

Induction of ovulation in women with PCOS by clomiphene citrate 100 mg once daily campared with induction of ovulation by letrozole 2.5 mg daily showed clear superiority of letrozole, higher endometrial thickness, higher ovulation rate (82.4% vs 63.6%), higher pregnancy rates ranging from 15% to 33% compared to 9.1% with clomiphene citrate induction (20).

In agreement with our study, study of Al-Omari et al(21) showed that endometrial thickness was significantly greater with letrozole compared with anastrozole (mean ± SD, 8.16 ± 1.32 versus 6.53 ± 1.55 mm; $p < 0.001$), Both endometrium thickness and ovulation rates per cycle were significantly higher with letrozole compared with anastrozole (84.4% vs 60% and 18.8% vs 9.7%, respectively; $p < 0.05$ to be a significant result with effective therapy .

Sharing same points with results of our study ,a randomized study by Jamilian et al (22)in PCOS patients received chromium picolinate 200 ug over 2 months showed higher pregnancy rate compared to placebo: 16.7 % vs.3.3 % Respectively ,also prevalence of acne decreased

Conclusion:

Letrozole alone can be effectively used to manage cases of PCO. Adding chromium picolinate potentiates the therapeutic effect of letrozol on pituitary hormones (FSH and LH), reduction of body mass index, improvement of ovulation, normalization of Estrogen level, improvement of endometrial thickness related to ovulation, improvement of progesterone production after good ovulation, reduced fasting insulin and reduction of androgen levels.

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