Research Article

Low dose Aspirin with clomid in pco

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Abstract
polycystic ovary syndrome is the commonest cause of an ovulatory infertility, and is the most common endocrine abnormality in reproductive age women.IN these study we try to evaluate the efficacy of co-administration of low-dose aspirin and clomiphene citrate on ovulation rates, endometrial thickness and clinical pregnancy rates in cases of clomiphene resistant PCOS at Minia university hospital over one year. Our study include 81 patient diagnosed with PCOS. Color and power Doppler ultrasonography was performed before and after treatment. Induction of ovulation done by clomiphene citrate 150 mg tablets and accompanied by a daily oral low dose of aspirin (75mg). Folliculometry, Endometrial thickness and appearance were assessed before and after treatment. We found that Dominant follicles of the studied group , Endometrial thickness and appearance and Clinical pregnancy rate were highly statistically significant differences between before and after treatment**.** Our clinical trial showed that low-dose aspirin administration results in high pregnancy rate. LDA seems to be a useful, effective, cheap and safe treatment in patients who undergo ovulation induction.
Key words: Low dose aspirin, clomiphene citrate, power Doppler, PCO.

Introduction
The polycystic ovary syndrome (PCOS) is the commonest cause of an ovulatory infertility accounting for approximately 75% of the cases. (Elkhateeb et al., 2017). PCOS is the most common form of WHO type II anovulatory infertility, and is the most common endocrine abnormality in reproductive age women. (Homburg et al., 2005)

Clomiphene citrate (CC) is still holding its place as the first-line therapy for ovulation CC is capable of inducing a discharge of FSH from the anterior induction in these patients . Pituitary and this is often enough to reset the cycle of events leading to ovulation. This is achieved indirectly, through the action of CC, a non-steroidal compound closely resembling an estrogen, in blocking hypothalamic estrogen receptors, signaling a lack of circulating estrogen to the hypothalamus and inducing change in the pattern of pulsatile release of GnRH (Elkhateeb et al., 2017).

A prospective cohort study of CC resistant PCO patients treated with an extended duration of CC administration to overcome anovulation. Prolonged duration of CC is safe, effective and minimal side effect (Elkhateeb et al., 2017).

Clomiphene resistance defined as failure to ovulate after receiving 150 mg of CC daily for 5 days per cycle, for at least three cycles, is common and occurs in approximately 15 to 40% in Failure of CC to induce ovulation or CC resistance, is unpredictable and women with PCOS (National Collaborating Centre for Women's and Children's Health, National Institute, 2005). Foremost unexplainable event, some studies showed thatit is more likely in patients who are obese, insulin resistant and hyperandrogenic a genetic predisposition was suggested (Elkhateeb et al., 2017).

Standard practice is to administer CC for 5 days from the second or third day of the menstrual cycle, starting with 50 mg/day and increasing up to 250 mg. However, managed care studies have shown that the most effective dosage 100–150 mg/day and over 75% of ovulations occur within these dosages. After six to nine cycles of treatment with CC cumulative pregnancy rates reach 70–75% (Elkhateeb et al., 2017).

Low-dose aspirin (LDA) has been shown to increase both ovarian and uterine blood flow through inhibiting the synthesis of thromboxane A2 resulting in vasodilatation and inhibition It was suggested that LDA treatment significantly improves of platelets aggregation (Aref et al., 2019).

Ovarian responsiveness, folliculogenesis, uterine and ovarian blood flow velocity, implantation and pregnancy rates (Younis et al., 2011).

Significant number of anovulatory infertile women is resistance to anti-estrogens treatment and need another treatment modality to improve results before shifting to other expensive protocols. In the literature, LDA could be added to CC as an adjuvant modality (Aref et al., 2019).

Meanwhile; the co-administration of both clomiphene citratrate and low-dose aspirin has not been reported in the field of infertility up till now. This has inspired us to evaluate the efficacy of this novel protocol on ovulation rates, endometrial thickness and pregnancy rates in infertile anovulatory PCOS women (Aref et al., 2019).

The availability of an adequate vascular supply may play a major role in the regulation of Color and especially power Doppler haveb been normal follicular growth and ovulation shown to assess the microvascular network within the ovarian stroma. As well, they have been revealed to have a fundamental role in the study of stromal vasculature in both normal and polycystic ovaries (Younis et al., 2011).

Patients and Methods

This clinical trial include the women presented to the outpatient clinic of our department with an ovulatory infertility either primary or secondary due to Pco after exclusion of other factors of infertility. The study included women in the reproductive age (20–35 years). patients were diagnosed to have PCOS based on the presence of at least 2 of the following criteria based on Rotterdam criteria *(Rotterdam ESHRE/ ASRM: 2004).* Explanation of the procedure to all women participating in the study. A written consent was taken from all patients before starting the study with counseling about risk and benefit of study. Patients were subjected to Complete history taking and Examination General, abdominal and local examination. Bimanual pelvic examination of both adenexa, and uterus for detection of any abnormality of female genitalia. Transvaginal ultrasound examination was done using [GE Voluson machine] with 7.5 MHZ vaginal transducer to evaluate the uterus, ovaries and to confirm the diagnosis of pcos. The ovarian volume, stromal thickness and the number and arrangements of follicles were evaluated. Ovarian Doppler Studies: Basal stromal ovarian blood flow studies were performed in before starting treatment, and following the treatment.

Color and power Doppler ultrasonography was performed with use of a two-dimensional endovaginal probe of 5 to 9 MHz frequency. Areas of maximum color intensity, representing the greatest Doppler frequency shifts, were selected for pulsed Doppler examination. Peak systolic blood flow velocity waveforms were thus detected, and optimal flow velocity waveforms were selected for analysis. A recording was considered satisfactory for measurement when there were at least three equally intense waveforms in a row. For each woman, the average of each index in both ovaries was calculated and used for statistical analysis. An ovary that did not show waveforms for accurate analysis, including by power Doppler sonography, was concluded to be an ovary with no detectable flow.

Treatment protocol include Induction of ovulation had been by cc 150 mg tablets once daily started from 2nd day for five days . A daily oral low dose of aspirin (75mg) at the same time of induction with cc and to the end of the cycle. Folliculometry was performed by transvaginal sonography (TVS) every other day starting from the 9th day of menstrual cycle. Endometrial thickness and appearance (triple layer or not) were also assessed. Power Doppler for reassessment of overian vascularity. Human Chorionic Gonadotropin at a dose of 5000 IU was administered intramuscularly when at least one follicle with a mean diameter 18 mm. Timed intercourse was advised 24 to 36 h after (HCG) injection. All participants received 400 mg of progesterone vaginally once daily from

HCG injection and for two weeks. Patients advised to continue aspirin treatment till a positive pregnancy test or three successive cycles of treatment. The study outcome measures included: Number of mature follicles, endometrial thickness and shape and pregnancy rates after treatment.

Results

Table (1): Comparison of patient's ultrasound data before and after TTT .

	Before TTT		After TTT		P value
	No.	%	No.	%	
Ovarian Volume					
Range	22-38		23-35		0.968
Mean±S.D.	29.53±3.423		29.48±3.511		
Dominant follicle					
<1.8 cm	81	100	20	24.7	<0.001*
≥1.8 cm	0	0	61	75.3	
Range	0.2-0.9		1-3		<0.001*
Mean±S.D.	0.53±0.229		1.63±1.167		
Uterus size					
Range	24.8 – 54.5		25.5 – 54.7		1.000
Mean±S.D.	38.96 ± 5.876		39.12 ± 5.964		
Endometrial thickness (mm)					
Range	2-7		2-8		<0.001*
Mean±S.D.	4.42±1.967		6.28±1.222		
Endometrial thickness shape					
Non-Triple Layer	14	17.3	31	38.3	0.005*
Triple Layer	67	82.7	50	61.7	

Regarding overian volume {cm3} :Before treatment ranged between 22 to 38 with a mean value of 29.53±3.423. After TTT volume ranged between 23to 35 with a mean value of 29.48±3.511 with no statistically significant differences between before and after TTT.

Regarding dominant follicle {cm} : Dominant follicles of the studied group before TTT show that all follicle was <1.8 cm After TTT 61(75.3%) had follicles ≥18 mm. Follicles before TTT was ranged between 0.2 – 0.9 cm with a mean value of 0.53±0.229 % while after TTT it was ranged between 1 – 3 cm with a mean value of 1.63±1.167 % there were highly statistically significant differences between before and after TTT.

Regarding uterian volume {cm3} : Before TTT uterian size was ranged between 24.8 – 54.5 with a mean value of 38.96 ± 5.876% . After TTT was ranged between 25.5 – 54.7 with a mean value of 39.12 ± 5.964 % with no statistically significant differences between before and after TTT.

Regarding Endometrial Thickness {mm}: Before TTT was ranged between 2–7 with a mean value of 4.18±1.574% . After TTT was ranged between 2 – 8 with a mean value of

6.28±1.222% which were highly statistically significant differences between before and after TTT with P value (<0.001).

Regarding Endometrial appearance: Endometrial thickness appearance of the studied group show that 14(17.3%) with non-triple layer and 67(82.7%) with triple layer before TTT.

After TTT results show that 31 (38.3%) with non-triple layer and 50 (61.7 %) with triple layer endometrial thickness shape which were highly significant which were highly statistically significant differences between before and after TTT with P value (<0.005).

Table (2): **shows clinical pregnancy rate of the studied group:**

Clinical Pregnancy Rate	Number	Percent
No	48	59.3
Yes	33	40.7
Total	81	100

Table (3): Distribution of studied sample according to patient's Early follicular stromal ovarian blood flow.

Early follicular stromal ovarian blood flow	Min. – Max.	Mean ± S.D.
PSV	7 – 24	15.31±5.255
PI	0.21 – 1.97	0.98±0.532
RI	0.40 – 0.80	0.60±0.109
S/D ratio	1 – 4	2.58±1.071

According to this table PSV follicular stromal ovarian blood flow was ranged between 7–24 cm/s with a mean value of 15.31±5.255 cm/s. PI follicular stromal ovarian blood flow was ranged between 0.21–1.97 with a mean value of 0.98±0.532. RI follicular stromal ovarian blood flow was ranged between 0.40–0.80 with a mean value of 0.60±0.109. S/D ratio follicular stromal ovarian blood flow was ranged between 1–4 with a mean value of 2.58± 1.071.

Discussion

Polycystic ovary syndrome (PCOS) is the most prevalent endocrine disorder among premenopausal women Globally, the incidence of this syndrome varies, ranging from 6% to 20% depending on the diagnostic criteria applied, with higher prevalence in overweight women. PCOS is a complex and heterogeneous endocrinopathy characterized by a constellation of symptoms and clinical features, including hyper androgenism (clinical or biochemical), ovarian dysfunction (menstrual irregularities), and polycystic ovarian morphology. Currently, there are several diagnostic criteria for PCOS that utilize different combinations of these clinical traits. According to the Rotterdam criteria, the most widely used for the clinical diagnosis of PCOS is defined by at least two of the three mentioned clinical features. PCOS is considered the leading cause of anovulatory infertility and is therefore clinically associated with defuncdibility (Sanchez-Garrido and Tena-Sempere 2020).

Our study aims to evaluate the efficacy of co-administration of low-dose aspirin (LDA) and clomiphene citrate on ovulation rates, endo-

metrial thickness and clinical pregnancy rates in cases of clomiphene resistant PCOS.

The study included 81 women presented to the outpatient clinic of our department with an ovulatory infertility due to Pcos after exclusion of other factors of infertility.

In 1991, Kurjak et al., proposed that blood flow parameters surrounding the follicle could be used as an index to evaluate follicular growth, maturation and ovulation. The function status of a reproductive organ is closely associated with its angiogenesis and hemodynamic performance. The blood circulation status, amount of tissue blood perfusion and blood flow resistance are prerequisite conditions for the growth and development of ovarian follicles. In a previous study, it was demonstrated that following the injection of hCG, the values of the ovarian artery blood flow indexes, PI and RI, predict the development of follicles, and are reliable indicators for evaluating the degree of maturity of follicles and the quality of the ova .Low-dose aspirin (LDA) has been shown to increase both ovarian and uterine blood flow through inhibiting the synthesis of thromboxane A2 resulting in vasodilatation and inhibition of platelets aggregation.

It was suggested that LDA treatment significantly improves ovarian responsiveness, folliculogenesis, uterine and ovarian blood flow velocity, implantation and pregnancy rates in IVF patients (Dhaliwal et al., 2011).

Aspirin has ameliorating effects against polycystic ovary syndrome via anti-inflammatory and hormonal modulatory pathways (Olaniyan et al., 2020).

Endometrial thickness is one of the essential factors in determining the possibility of success in any ovulation induction program. Of note, at least an endometrial thickness of 8 mm or more is essential for implantation, also preclinical abortions markedly increased in patients whose endometrial thickness was less than 8 mm on the day of HCG administration (Chia-Woei et al., 2008).

In the present study, the co-administration of LDA and cc appears to be associated with more favorable outcomes compared to cc alone in ovulation induction. The mean number of follicles, endometrial thickness, and cumulative clinical pregnancy rates are all improved with the addition of LDA to cc.

Our study results Outcome:

Dominant follicles of the studied group before TTT show that all follicle was <1.8 cm while after TTT 61(75.3%) had follicles ≥18 mm. Follicles before TTT was ranged between 0.2 – 0.9cm with a mean value of 0.53±0.229% while after TTT it was ranged between 1–3cm with a mean value of 1.63±1.167 % which were highly statistically significant differences between before and after TTT with P value (< 0.001).

Endometrial thickness before TTT was ranged between 2-7mm with a mean value of 4.18± 1.574% while after TTT it was ranged between 2-8mm with a mean value of 6.28±1.222% which were highly statistically significant differences between before and after TTT with P value (< 0.001).

Endometrial thickness appearance of the studied group show that 14(17.3%) of patients with non-triple layer and 67(82.7%) with triple layer shape, while after TTT results show that 31 (38.3%) with non-triple layer and 50(61.7%) with triple layer shape which were highly statistically significant differences between before and after TTT with P value (< 0.05).

Early follicular stromal ovarian blood flow data shows: PSV follicular stromal ovarian blood flow was ranged between 7–24 cm/s with a mean value of 15.31±5.255 cm/s. PI follicular stromal ovarian blood flow was ranged between 0.21–1.97 with a mean value of 0.98±0.532. RI follicular stromal ovarian blood flow was ranged between 0.40–0.80 with a mean value of 0.60±0.109. S/D ratio follicular stromal ovarian blood flow was ranged between 1–4 with a mean value of 2.58±1.071.

Clinical pregnancy rate of the studied group show that 33 (40.7%) while 48 (59.3) were non pregnant of total number 81 patient.

In another study of Aref, et al., 2019 which was a randomized clinical trial, aimed to evaluate the efficacy of co-administration of low-dose aspirin (LDA) and tamoxifen on ovulation

rates, endometrial thickness and clinical pregnancy rates in an ovulatory PCOS women the endometrial thickness was significantly thicker in the study group {9.6 mm vs. 7.8 mm} in the control group, the triple endometrial appearance was more evident in the study group (87% vs. 83%) but without a significant statistical difference. Most importantly, cumulative clinical pregnancy rate after a maximum of 3 cycles of OI was significantly higher in the study compared to the control group (37.2% versus 22.3% respectively.

In agreement with our findings, the study of Aref, et al., 2019, reported that follicles ≥18 mm was with Mean± SD was 1.4 ±0.8.
In Zhao, Du et al., 2014 which aimed to investigate the clinical value of low‐dose aspirin in combination with Tiao Jing Cu Yun pills in patients with polycystic ovary syndrome (PCOS) by measuring follicular peripheral blood flow parameters and the clinical efficacy. The subjects in the experimental group were treated with letrozole in combination with aspirin and Tiao Jing Cu Yun pills, and the control group was treated with letrozole alone, The control group pregnancy rate was only 27.50%, while that of the experimental group of patients who were reintegrated in the second cycle was 37.93%, which was significantly higher ($P<0.05$).
Rubinstein et al., 1999 reported that LDA treatment significantly improves ovarian responsiveness, uterine and ovarian blood flow, and implantation and pregnancy rates in their patients. Rubinstein et al., performed their study using low-dose aspirin (100 mg /day) combined with controlled ovarian hyperstimulation for IVF protocol.

In a prospective and randomized trial, Hsieh et al., 2000 demonstrated higher pregnancy rate and better endometrial pattern in patients with thin endometrium after aspirin administration. However, no evidence of improvement was detected in the endometrial thickness, uterine artery PI and RI and spiral artery.

In contrast the Madani, Ahmadi et al., 2019 study, which was a pilot randomized, double-blind placebo-controlled trial aimed to evaluate the effect of adjuvant low-dose aspirin therapy on clinical pregnancy rate and uterine perfusion in women undergoing frozen-thawed embryo transfer (FET) cycles.

Another studies of Haapsamo et al., 2009; Weckstein et al., 1997 showing no significant differences in endometrial thickness between low-dose aspirin-treated women and control group during IVF or ICSI treatment.

Interestingly; the present study revealed that clinical pregnancy of the studied cases showed that 33(40.7%) were pregnant, So, we can explain the high pregnancy rate in the study as a result of improved endometrial thickness with the use of LDA. In addition, prostaglandins (PGs) stimulate inflammatory cells and the release of interleukins, which produce inflamemation and in turn may diminish the implantation rate. PGF2a stimulates uterine contraction, which may also affect implantation. LDA may avoid these negative effects by irreversibly inhibiting cyclooxygenase, which blocks the synthesis of prostaglandins (Vane and Botting, 1992).

Conclusion

In conclusion, our clinical non-controlled trial showed that low-dose aspirin administration results in slightly high pregnancy rate, our study shows promising results that highlight the potential role for LDA with clomiphene citrate as an additional co-factor to improve ovarian response; prepare the endometrium to increase the chances of pregnancy. LDA seems to be a useful, effective, cheap and safe treatment in patients who undergo ovulation induction. Further wide scale researches are required to ensure the effectiveness of this co-administration as a novel protocol for ovulation induction.

Recommendations:

Further wide scale researches are required to ensure the effectiveness of this co-administration as a novel protocol for ovulation induction.

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