Original article

Melatonin enhances ovarian response in infertile women with polycystic ovary syndrome

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Abstract:

Background: Polycystic ovary syndrome (PCOS) is a common endocrine disorder in women of reproductive age. Anovulation, decreased Oocyte quality and low endometrial receptivity are the cause of infertility in women with PCOS. Anovulation is the consequence of hyperandrogenism, insulin resistance. Furthermore, the reactive oxygen species (ROS) induce oxidative stress which may be responsible for poor Oocyte quality. Melatonin is a documented powerful free radical scavenger and broad-spectrum antioxidant. Current evidence suggests that melatonin involves in ovarian physiology including follicular development, ovulation, and oocyte maturation. Present study was tried to evaluate the effects of melatonin on biochemical parameters as well as outcomes of ovulation induction by letrozole in infertile women with polycystic ovary syndrome. ***Method:*** This study including 74 women of Polycystic Ovary Syndrome (PCOS) with infertility. Intervention group received melatonin 3 mg at bed time for 8 weeks as pretreatment. Serum luteinizing hormone (LH), testosterone, anti mullerian hormone (AMH), fasting insulin, oral glucose tolerance test (OGTT) were measured at baseline and after 8 weeks. Both intervention and control group were received ovulation induction for 3 cycles by Letrozole (5 mg from cycle days 2 to 6). Intervention group continued melatonin until mature follicle achieved. The primary outcomes were biochemical changes by serum luteinizing hormone (LH), testosterone, anti mullerian hormone (AMH), fasting insulin, oral glucose tolerance test (OGTT) and ovarian responses by number of mature follicles, endometrial thickness and ovulation rate. Secondary outcome was pregnancy rate. ***Result:*** Melatonin treatment for 8 weeks significantly decreased testosterone (P <0.01) serum luteinizing hormone (<P.001), HOMA IR (P<0.01) and glucose tolerance (P<0.01). The change of anti mullerian hormone was not significant (>0.05). There was significant difference in number of mature follicles (< 0.01), mean endometrial thickness (P<0.01). The risk ratio (RR) of ovulation rate was 1.34(0.09-1.68) and pregnancy rate was 2.55 (.37-3.51). The risk ratio (RR) of pregnancy rate in relation to AMH level was 1.12(0.05-1.79) in ≤8ng/ml group and 8.65(0.25-9.59) in ≥8ng/ml group which was significant. ***Conclusion****:* After 8 weeks pretreatment and 3 cycle's co treatment with ovulation induction by letrozole, melatonin seems to provide improved biochemical and ovarian response. Based on these results, melatonin could be considered as a potential therapeutic agent for infertile women with polycystic ovary syndrome.

Keywords: Polycystic ovarian Syndrome; melatonin, letrozole; Ovulation Induction.

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Introduction:

Polycystic ovary syndrome (PCOS) is the largest single cause of anovulatory infertility (80%) [1]. It affects 6-10% of women at reproductive age[2]. According to The Rotterdam Criteria, 2003 at least two of the following three features should be present for proper polycystic ovary syndrome diagnosis: oligoovulation and/or anovulation; clinical or biochemical hyperandrogenism; polycystic appearance of ovaries in ultrasonography.

Women with polycystic ovary syndrome usually present with infertility and menstrual cycle irregularities at reproductive age. In addition to these, it is associated with higher risk of metabolic disorders including insulin resistance, type 2 diabetes mellitus, increased oxidative stress, cardiovascular disease, liver disease, and endometrial cancer[3].

Changing lifestyles, such as nutritional counseling and weight loss are the necessary steps of all treatment modalities for polycystic ovary syndrome (PCOS) women. Insulin sensitizers, oral ovulation inducing agents, gonadotropins & laparoscopic ovarian drilling are also used for treatment of anovulatory infertile PCOS woman. Many questions remain unanswered about the pathobiology and treatment strategies of PCOS despite many advances over the past decades.

Melatonin (N-acetyl-5-methoxytrypamine) is a 'chronobiotic' hormone that was first isolated in the 1950s from bovine pineal gland. It was also found in gastrointestinal tract, skin, retina, bone marrow, lymphocytes and the female reproductive organ including the follicular cells, oocytes, and cytotrophoblasts[4]. Melatonin production and secretion is regulated by photoperiod and effects mediated via receptors[5]. It has antioxidant, anti-angiogenic, immunomodulatory, and oncostatic effects[6].

In PCOS patients, melatonin level in serum is usually higher than in healthy women and lower in follicular fluid, which is considered as a sign of diagnosing PCOS[7]. During the night, PCOS women had higher levels of urinary 6-sulfatoxymelatonin (aMT6s), the most important metabolite of melatonin. The production of higher amounts of melatonin is probably for neutralizing increased amount of reactive oxygen species (ROS). Due to lower intra-follicular melatonin concentration oxidative stress increases, resulting in follicular atresia and ultimately causes anovulation or poor oocyte quality[7].

Some growing evidence suggests that administration of melatonin in poly cystic ovary syndrome can improve metabolic function and ameliorate the hormonal picture significantly. It has receptor mediated effects on metabolism. It's reducing effects on hepatic gluconeogenesis, improvement of glucose uptake by adipocytes and skeletal muscle cells, and reduction in insulin levels, may be the cause of metabolic improvement[8]. Melatonin is associated with an increased risk of type 2 DM when night-time is reduced.[9] PCOS patients with higher LH:FSH ratios having significantly lower melatonin levels in follicular fluid, indicates the inverse relationship of LH:FSH ratio and melatonin secretion[10].

Melatonin plays an important role in scavenging reactive oxygen species (ROS) [4]and correction of hyperandrogenism which promote follicular maturation and ovulation[10] as well as protect corpus luteum[11]. Supplementation of melatonin can protect oocytes from oxidative stress leading to the successful reproductive outcomes in women of PCOS undergoing artificial reproductive technology (ART) [12].

Letrozole, an aromatase inhibitor is a good option for ovulation induction due to its short half-life and negligible side effects. Long term combined use of melatonin and letrozole on endometriotic rat model was well tolerated with no significant side effects[13].

The aim of the present study is to investigate the effectiveness of melatonin administration on biochemical features of women affected by polycystic ovary syndrome. In particular, the primary end point of the study was to explore the possible role of melatonin co treatment with letrozole on ovulation and pregnancy rate.

Materials and Method

This study was performed on 74 diagnosed cases of PCOS women with infertility selected for ovulation induction from outpatient department of Reproductive Endocrinology and Infertility Department at Bangabandhu Sheikh Muzib Medical University (BSMMU), Dhaka. The randomization was done by computer generated random table without blinding.

The sample size was 42 in each group considering type 1 error of 5%, a study power of 80.74%, drop out 10%. Due to several times lockdown in corona pandemic situation during the study period, the defined sample size could not achieved.

The participants were included in this study with the following criteria: diagnosed case of PCOS

patients according to Rotterdam criteria, Age 18 to 35 years, BMI ≤30 kg/m^2, and serum AMH level ≥4 ng/ml. The following clinical findings were considered as exclusion criteria: hypothyroidism, hyperprolactinemia, diabetes mellitus (fasting glucose ≥ 7mmol/l), bilateral tubal block, abnormal semen parameters, any history of taking insulin sensitizer, known sensitivity to melatonin and concurrently taking medications known to interact with melatonin (antidepressants, antiepileptics, or hypnotics).

Study Procedures:

Total 74 participants were randomly allocated in two groups. Forty participants received melatonin as pretreatment for 8 weeks than cotreatment during ovulation induction by letrozole as the intervention group and 34 participants received ovulation induction by letrozole as the control group.

An informed written consent was taken from all participants after proper counseling. Data were collected through interview, physical examinations and laboratory investigations. A full assessment, which included demographic information, BMI, infertility duration, type of infertility, baseline infertility investigations and hormonal assays like serum luteinizing hormone (LH), follicle stimulating hormone(FSH), thyroid stimulating hormone(TSH), anti mullerian hormone(AMH), Prolactin, total testosterone, fasting insulin and Oral glucose tolerance test (OGTT) was done. The clinical and laboratory workup was conducted during the early follicular phase (days 2-6) of the menstrual cycle.

Follicle stimulating hormone (FSH), testosterone (T), and luteinizing hormone (LH) were measured by using electro chemiluminescence immunoassay (Roche diagnostics GMbH, Mannheim, Germany). Serum AMH level was measured by using a second-generation enzyme linked immunosorbent assay (ELISA) (AMH Gen II ELISA: Beckman Coulter and R&D Systems). The analytical sensitivity of this assay was 0.014ng/ml. Intra and inter-assay coefficients of variation were ≤12.3 and ≤14.2% respectively. Oral glucose tolerance test (OGTT) with a 75-g glucose load was carried out in all participants after overnight fasting for at least 8 hours; fasting plasma glucose (FPG) and plasma glucose 2 hours after OGTT (PG 2H-OGTT) were measured by the glucose oxidase method using a fully automatic biochemistry analyzer. Prediabetes and diabetes mellitus were diagnosed according to criteria described by the American Diabetes Association[14]. Impaired Fasting Glucose is defined as Fasting Plasma Glucose levels between 100 and 125 mg/dL (between 5.6 and 6.9 mmol/L) and Impaired Glucose Tolerance as 2-hours plasma glucose during 75-gOGTT levels between 140 and 199 mg/dL (between 7.8 and 11.0 mmol/L). Both impaired fasting glucose (IFG) And impaired glucose tolerance (IGT) were counted as impaired OGTT in this study. Evaluation of insulin sensitivity was made by the homeostatic index (HOMA), which was calculated as follows: HOMA = ¼ (fasting insulin [mU/mL] × fasting glucose [mmol/L] /22.5). Fasting insulin was measured from at least 8 hours overnight fasting blood samples.

Treatment was started with melatonin 3 mg (Tab FILFRESH 3 mg, Square Pharmaceuticals Limited) at bed time for the intervention group irrespective of cycle as pretreatment for 8 weeks. Then they had follow up measurement of body weight, serum AMH, LH, total testosterone, fasting insulin and OGTT. After that they were given letrozole 5 mg at night from day 2 of menstrual cycle or withdrawal bleeding to day 6, continuing melatonin 3mg at bed time until mature follicle (18mm or more from day 12 to day 16 of menstrual cycle) was achieved. The patient was asked to continue melatonin even if there was no dominant follicle.

The patients who were allocated to control group were given letrozole in same dose and duration as in melatonin group. The study included ovulation induction up to 3 cycles.

Ovarian response was assessed by follicular tracing with transvaginal sonography (TVS) till the appearance of preovulatory follicle (mean diameter ≥18mm) between day 12 and day 16 of menstrual cycle. The endometrial thickness was measured as well. Transvaginal pelvic ultrasound was performed on each patient using a 6.5 MHz transvaginal probe (Mindray DP-2200plus) for baseline and folliculometry. In both the groups inj. HCG 5000 IU was given to women when follicular size was ≥ 18mm and timed intercourse was advised every other day from the day of HCG administration. Serum progesterone levels were measured once between days 21 to 23 of the cycle.Serum progesterone >3 ng/ml considered as ovulation in this study[15].

Human chorionic gonadotropin (β-HCG) was measured on day 16 after HCG triggering or any time after missed period. Positive urinary pregnancy test or gestational sac on ultrasound was also considered

as pregnancy in this study. If there was no evidence of pregnancy, the patient repeated the same procedure in the next cycle.

Procedures of data analysis

Statistical analysis was carried out by the Statistical Package for Social Sciences 23.0 for Windows (SPSS Inc., Chicago 11 and USA). Quantities of data were expressed as means ± SD and qualitative data expressed as percentages. Paired and unpaired Student´s t-test, Chi-square test and Fisher's exact test were used to test of significance.

Ethical Implications

The objective of the study along with its procedure, alternative diagnostic methods, risk and benefits was explained to the patient in details, in easily understandable local language and then voluntary informed written consent was taken from the patient before collecting data. Privacy, anonymity and confidentiality were maintained during the procedure.

Results

Among 40 woman of melatonin group, one was lost follow up in 3rd cycle and among 34 women of letrozole only group,2 in first cycle and 4 in 2nd cycle was lost follow up.

The mean age of the participants in the present study were 25.20±3.58 years in melatonin group and 25.18±3.45 years in control group respectively, ranging from 22-34 years. More than half of the patients belonged to age group 20 to 24 years. Baseline characteristics of the participants did not show any significant differences between two groups. Table 1 summarizes their baseline characteristics.

Table 1: Baseline demographic, clinical, hormonal and metabolic features of the participants.

Variables	Melatonin and letrozole N=40	Letrozole only N=34	P Value
Age	25.20±3.58	25.18±3.45	0.838
BMI (Body Mass Index kg/m^2)	27.64±2.47	27.00±2.26	.196
Duration of infertility (Year)	4.06±3.12	4.34±3.26	.964
Serum AMH (ng/ml)	9.62±5.42	10.10±4.83	.315
LH (IU)	8.82±3.73	8.08±2.92	.990
Serum Testosterone Ng/ml	50.12±25.65	44.79±29.16	.191
HOMA IR	4.08±1.51	3.65±1.54	.091
	Types of infertility (%)		
Primary infertility	32(80)	27(79.4)	.360
Secondary infertility	8(20)	7(20.6)	
Hyperandrogenism (%)	31(77.5)	23(67.6)	.519
Anovulation (%)	25(62.5)	19(55.88)	.671
PCO in ultrasonogram (%)	32(80)	28(82.35)	.069

Data are presented as Mean±SD or n (%).

The hormonal and metabolic features of melatonin group (n=40) at baseline and after 8 weeks of therapy showed significant changes. There was significant reduction of serum total testosterone (P =.000), serum LH level (P= .007), HOMA IR (P=.00), OGTT (P=.007) after 8 weeks pretreatment with melatonin. The change in serum AMH level (P=.178) was not significant(Table 2).

Flow chart of study procedure

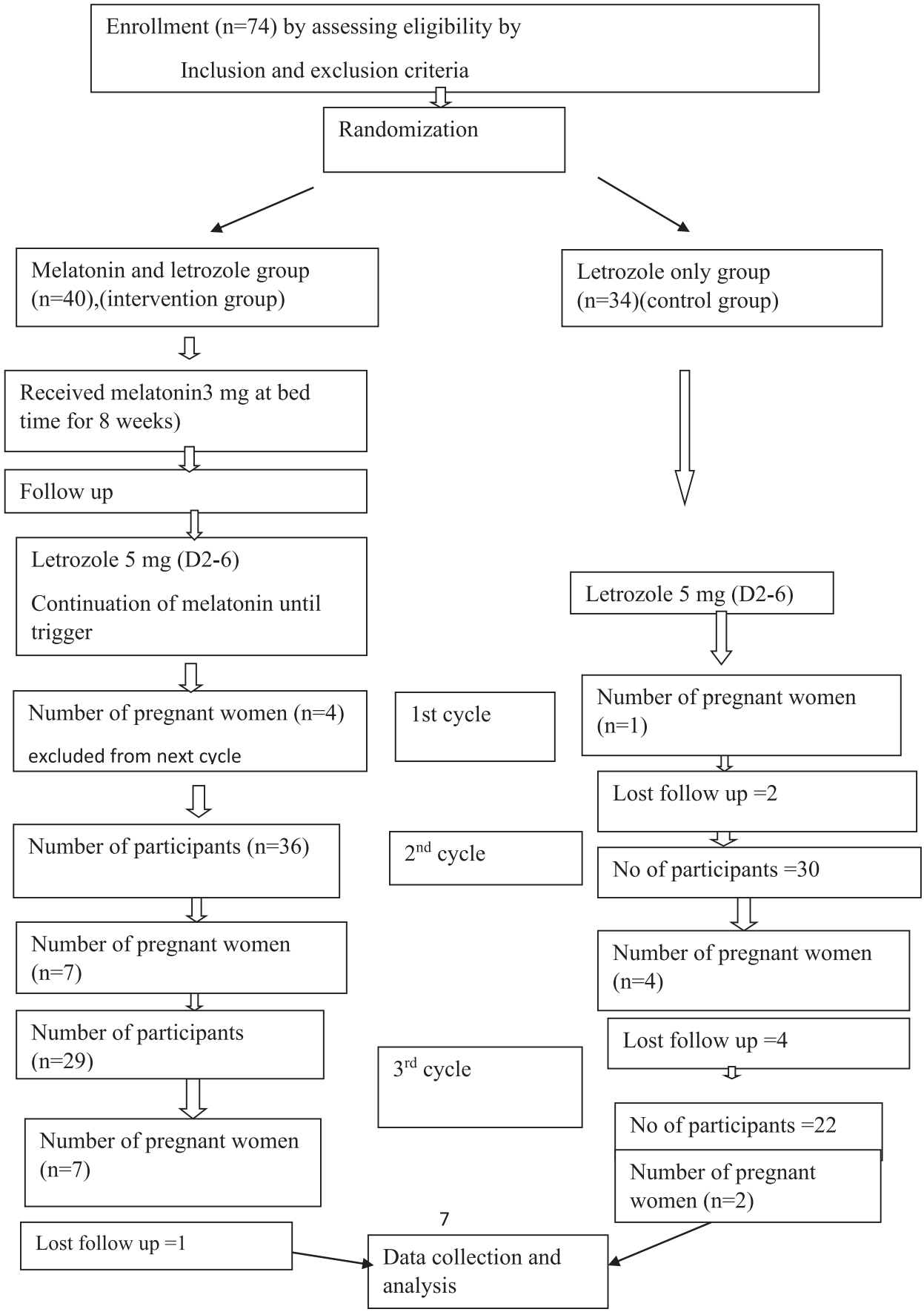

Table 2. Biochemical responses between base line and post treatment parameters in intervention group.

	Pretreatment	Post treatment	P value
Serum LH (mIU/ml)	8.82±3.73	6.00±3.97	.007
Serum AMH (ng/ml)	9.62±5.42	9.12±6.62	.178
HOMA IR	4.08±1.15	2.94±1.191	.000
Serum testosterone (ng/dl)	50.12±25.65	36.58±19.59	.000
OGTT impaired (%)	26(65)	10(25)	.007

Data are presented as Mean±SD or n (%).

Table 3 shows the outcome of ovulation induction. The number of mature follicles obtained per cycle, dominant follicle size, endometrial thickness and midluteal progesterone were significantly higher in melatonin-letrozole group compared to letrozole only group.

Ovulation rate was 80.3% and 59.53% in melatonin and control group respectively. The Risk Ratio (RR) of ovulation rate in melatonin group was 1.34 (95% CI .09-1.68).

Table 3. Comparison of outcome of ovulation induction between melatonin-letrozole and letrozole only groups.

Variables	Melatonin and letrozole N=40	Letrozole only N=34	P value
Number of mature follicles	1.90±0.92	1.02±0.90	0.000
Mean size of dominant follicle (mm)	19.43±1.72	16.96±5.27	0.017
Endometrial thickness(mm)	8.27±1.16	7.68±0.89	0.044
Serum progesterone	21.9±14.6	13.2±9.9	0.000

Data are presented as Mean±SD Pregnancy rate in melatonin group was 45% versus 20.58% in control group. The Risk Ratio (RR)(95% confidence interval CI) was 2.18(0.37-3.51) indicating an association of melatonin treatment with increasing pregnancy rate. (Table: 4)

Data are presented as n (%).

Table 4: Risk Ratio (RR)(95% CI)of Ovulation and pregnancy rate per cycle in between two groups.

Parameters	Melatonin and letrozole N=40	Letrozole only N=34	RR (95% CI)
Ovulation rate	(80.3%)	(59.52%)	1.34 (.09-1.68)
Pregnancy rate	(45%)	(20.58%)	2.18(.37-3.51)

To determine the relationship of pregnancy rate and serum AMH level we divide the whole study population into two groups, which are serum AMH ≤8 ng/ml and >8 ng/ml group. The risk ratio (95%CI) of pregnancy rate was 1.12(.05-1.79infirst group but interestingly the risk ratio (95%CI) in second group was 8.65(0.25-9.59) in 2nd group, where the mean anti mullerian hormone (AMH) is 13.39± 5.34 and 12.02± 4.44 in Melatonin-letrozole and letrozole only group respectively. This result indicates that the melatonin treatment has strong relationship with increasing pregnancy rate in PCOS woman even with high anti mullerian hormone (AMH) (table: 5).

Discussion:

This study was aimed to observe the effect of melatonin on biochemical response in terms of serum total testosterone level, serum LH level, HOMA IR, OGTT and ovarian response in terms of size of dominant follicles, number of mature follicles, endometrial thickness, ovulation rate and pregnancy rate in infertile PCOS women.

This study showed significant decrease in serum total testosterone levels after 8 weeks of melatonin treatment which is supported by a 6 months prospective cohort study by Tagliaferri et al[16], and a 12 weeks study by Jamilian et al[17]. Serum LH level was significantly reduced in comparison to baseline after 8 weeks treatment with melatonin in our study,

Table 5: Pregnancy in relation to AMH level in melatonin-letrozole and letrozole only groups.

AMH	Melatonin-letrozole		Letrozole only		Risk ratio (95% CI)
	Mean AMH	Pregnancy rate	Mean AMH	Pregnancy rate	
≤8 ng/ml	5.85± 1.23	45% (9/20)	5.60± 1.32	40% (6/15)	1.12(.05-1.79
>8 ng/ml	13.39± 5.34	45% (9/20)	12.02± 4.44	5.2% (1/19)	8.65(.25-9.59)

Data are presented asMean±SD or n (%).

similar to the findings of a prospective study for 8 weeks by Huda et al.,2018[18]. Changes in Anti-Mullerian hormone level were not significant after 8 weeks of melatonin treatment in our study, on the contrary it was significantly reduced after 6 months of treatment in the study by Tagliaferri et al[16]. The difference in duration of treatment may be the cause of discrepancy. HOMA-IR was significantly decreased in this study, a finding supported by a clinical trial conducted for 12 weeks by Hang et al[19]. Though Tagliaferri was showed no significant decrease of HOMA IR in their study. Normal BMI of recruited participants may be the possible explanation of the different finding.

Mean size of mature follicle, endometrial thickness and the number of mature follicles obtained per cycle was significantly improved in melatonin group in this study. We found no previous study on melatonin with letrozole used for ovulation induction. In a double blind RCT Mokhtari et al[20] investigated the effects of melatonin co treatment with clomiphene citrate on infertile PCOS women undergoing intrauterine insemination (IUI). Addition of melatonin significantly improved the sizes of follicles and total number of mature follicle as well as endometrial thickness in their study.

In our study, pregnancy rate was 45% versus 20.58 % [risk ratio 2.18(.37-3.51)] in melatonin and control group respectively. It was 30% in melatonin group, 18% in the control group (P=0.011) in Mukhtari et al study[20].

Ovulation per cycle was 80.3% and 59.53% in melatonin and control group respectively, the RR (95% CI) was 1.34 (.09-1.68). Eryilmaz et al, shows significant increase in the number of metaphase II (MII) Oocyte retrival and grade 1 embryos in IVF cycle after administration of 3-mg melatonin from days 3-5 until HCG injection[21].

We used AMH level 8 ng/mL as the cut off level to determine the pregnancy rate in relation to AMH. The pilot study by Xi et al determined that the AMH cut off value of 7.7 ng/mL has the sensitivity and specificity of 92% and 65% respectively, to determine ovarian responsiveness[22]. The risk ratio of pregnancy rate was 8.65 where the mean AMH was 13.39±5.34 and 12.02± 4.44 in Melatonin-letrozole and letrozole only group respectively. This result indicates that the melatonin can increase pregnancy rate in PCOS woman with high AMH.

There was no significant adverse effect of melatonin observed. Three patients had complaints of sleep disturbances which subsided after first week. Tagliaferri had similar findings[16].

There were some limitations of this study. Sample size was inadequate. Further TVS to see the collapsed follicle and fluid in cul de sac for ovulation confirmation was not possible due to pandemic situation.

Conclusion:

Infertile women with polycystic ovarian syndrome (PCOS), when treated with melatonin have significant improvements in biochemical parameters like reduced biochemical hyperandrogenism, decreased HOMA IR as well as correction of impaired oral glucose tolerance test (OGTT). There was significant improvement in ovarian response and the rate of pregnancies even in woman with high anti mullerian hormone. Thus, we concluded that there is a potential role of melatonin as a new therapeutic agent for infertile women with PCOS.

Source of fund: No

Conflict of interest

The authors declare they have no conflict of interest.

Ethical clearance:

The protocol has been approved in the Ethics Committee of Institutional Review board (IRB) Bangabandhu Sheikh Mujib Medical University (BSMMU) (Ethics committee registration no: 3144).

Authors' contribution:

Data gathering and idea owner of this study: Dr Sumaiya Akter

Study Design: Dr Sumaiya Akter

Data gathering: Dr Sumaiya Akter, Dr Chalontika Rani, Dr Shirin Jahan, Dr Sohely Nazneen.

Writing and submitting Manuscript: Dr Sumaiya Akter

Editing and approval of final draft: Professor Jesmine Banu, Professor Shakeela Ishrat, Dr Sumaiya akter

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