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Comparative Efficacy of Metformin vs. Pioglitazone alone and in Combination with High Dose Oral Cholecalciferol among Women with Polycystic Ovary Syndrome (PCOS): A Six-month Randomized, Open Label Study

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Abstract

Purpose: We aimed to evaluate the comparative efficacy of metformin and pioglitazone co-administered with high dose VD on clinical, biochemical, hormonal and insulin sensitivity parameters in women with PCOS.

Material and Methods: In this open label randomised study, a total of 136 women, aged 18-40 years, diagnosed with PCOS as per the AE PCOS 2009 criteria, were randomly assigned into four groups (n=34 per group). The four groups received Metformin-1gm/day alone, metformin-1gm/day+cholecalciferol4000IU/d,pioglitazone-30mg/day alone and pioglitazone-30mg/day+cholecalciferol-4000IU/d.The clinical, biochemical, hormonal and insulin sensitivity parameters were evaluated at 0, 3 months and 6 months after the intervention. Results: The mean age of subjects was 21.89±4.99 years with mean BMI of 22.19±3.24kg/m^2. The mean serum 25OHD level at the baseline in the metformin+VD and pioglitazone+VD group was 13.28±8.44ng/ml and 11.17±7.46ng/ml and it improved to 63.33±19.76ng/ml and 57.69±14.43ng/ml respectively after six months of intervention. The mean no. of cycles/year and Ferriman-Gallwey (FG) score improved in all four arms. Significant decrease in the total serum testosterone level was observed in the groups that received VD in addition to metformin and pioglitazone, the effect being more significant in the metformin+VD group (64.60±23.59ng/dl at 0 months to 41.24±12.83ng/dl after 6 months). Plasma glucose and insulin levels from a two-hour OGTT showed a significant improvement in subjects receiving VD in addition to pioglitazone.

Conclusion: Metformin and pioglitazone are equally efficacious for treatment of women with PCOS. High dose oral cholecalciferol co-administration significantly improved the metabolic outcomes among these women.

Key words: Metformin; Pioglitazone; PCOS; Insulin resistance; Vitamin D deficiency.

Introduction

Polycystic ovary syndrome (PCOS), is a heterogeneous, complex condition with triumvirate of clinical, endocrine and metabolic disturbances.[1] Globally 6-13% of the women in the reproductive

age group are affected, thereby making it the commonest endocrinopathy of these women.[2] The disorder is more prevalent in India with reports of higher proportion in urban population and existence of substantial phenotypic variations among regions.[3,4] The syndrome manifests with wide spectrum of presentations including oligo-anovulation (menstrual irregularities and infertility), hyperandrogenism (acne, hirsutism and alopecia), obesity, metabolic syndrome (MS), insulin resistance (IR), abnormal glucose tolerance (AGT), NASH, sleep apnea, psychiatric comorbidities etc.[5,6]

Lifestyle alteration aiming at weight reduction benefits most of the reproductive and metabolic features of PCOS and remains the cornerstone of its management.[7]Anti-androgens and insulin sensitizers have gained popularity as a treatment choice owing to their effect on hyperandrogenism and IR, the predominant pathogenic phenomena in PCOS.[8]Among insulin sensitizers, metformin has demonstrated usefulness in improving reproductive and metabolic features[9,10], however equivalent insulin sensitizing benefits of pioglitazone among women with PCOS[11] or non-PCOS subjects, were observed in the IRIS trial[12], making it a good alternative. In a small Indian study on PCOS women randomized to either receive pioglitazone or metformin, the former proved to be equally effective in restoring the insulin sensitivity and the menstrual cyclicity[13].

Vitamin D deficiency (VDD) has been linked to metabolic disarray among women with PCOS with many reports suggesting significant association between low serum 25OHD levels and PCOS phenotype.[14–18] Considering the fact that VDD has been linked to worsening of PCOS phenotype especially IR, AGT and hyperandrogenism, theoretically correcting VDD by replacing oral VD may result in improvement of these metabolic derangements. Accordingly, some published studies have shown significant improvement in the reproductive parameters and IR following VD supplementation[19,20], although others have failed to substantiate it.[21] The fact that VDD may modify or act as a confounder in assessing the response of PCOS women to treatment with insulin sensitizers, there is immense need to study the impact of these agents after rendering women with PCOS vitamin D replete.

In view of the above, we aimed to evaluate the comparative efficacy of metformin and pioglitazone alone and in combination with high dose oral vitamin D in women with PCOS.

Materials and Methods

This randomized, open label intervention trial was conducted among women with PCOS, aged 14–40 years, who attended Endocrinology outpatient clinic of All India Institute of Medical Sciences, (AIIMS), New Delhi enrolled between March, 2014 and October, 2014.

Clinical assessment: Women who attended the endocrine clinic for complaints of oligomenorrhea, unwanted hair growth, acne vulgaris, androgenic alopecia etc. were informed about the study and screened. Volunteers were asked to furnish details about menstrual history (age of menarche, duration and number of cycles/year), hyperandrogenism (duration and extent of unwanted hair growth, acne vulgaris, androgenic alopecia), weight gain, infertility, history of drug intake etc. A total of 235 women qualifying AE-PCOS 2009 criteria for the diagnosis of PCOS, were enrolled. Exclusions were made if the women were pregnant, had hyperprolactinemia, thyroid dysfunction, androgen-secreting tumours, Cushing's syndrome, Nonclassic congenital adrenal hyperplasia (NCAH), diabetes or AGT. Women refusing consent, with history of drug intake (vitamin D, calcium, oral contraceptive pills, antiandrogens, insulin sensitizers or drugs known to affect glucose, insulin, or vitamin D metabolism) in last 3 months were also excluded.

Physical examination: Anthropometric measurements such as weight, height, waist and hip circumference were measured and BMI was calculated. Systemic examination including measurement of blood pressure, quantitation of hirsutism, grading of acanthosis nigricans, acne vulgaris, and androgenic alopecia was undertaken for all participants. The modified FerrimanGallwey (FG)-score was used to assess the degree of hirsutism by a single observer and a score of 8 or above was taken as significant.

Laboratory assessment: OGTT was performed after 8-10 hours of overnight fast with 75 grams of anhydrous oral glucose load followed by sampling at 0, 60 and 120-minutes. The samples were aliquoted for biochemical estimations (calcium, phosphorous, liver function, kidney function, lipids) to be done on the same day while as aliquots for hormones (serum total T4, TSH, LH, FSH, PRL, total testosterone, 17-OHP and 25OHD were stored at -80ºC for assay at a later date. The PCO morphology was assessed with trans-abdominal ultrasonography performed in the follicular phase by a single sonologist using 7.5mHz probe (AlokaSSD-500, Tokyo, Japan) to quantitate ovarian

volume, count ovarian follicle number and assess thecal hyper echogenicity.

Assays: Biochemical parameters were estimated on a fully automated biochemistry analyser (DiaSysrespons®910, Germany) using standard commercially available kits with standard methodology. Hormonal assays including 25OHD were done by electrochemiluminescence immunoassay (Cobase411; Roche Diagnostics Limited, USA) except for plasma 17-OHP which was assayed by ELISA using commercial kits (Diagnostics Biochem, Ontario, Canada) according to supplier protocol.

Sample size Calculation: Sample size was calculated using nQuery software (Statsols, Boston MA). Considering type one error (α) as 0.05, power of study as 90% and attrition rate of 10% with reference from a recent study[22] that used two doses of cholecalciferol (1000 vs. 4000IU/day), we required around 35 subjects per arm.

Randomization: The subjects were distributed into four groups randomly using a computer generated random number allocation. After a subject met the inclusion criteria for the trial, an opaque envelope containing the random number was provided to each patient. The allocation concealment was maintained till all the parameters were available. After that all the participants, investigators as well as the research team were aware about the intervention.

Intervention: The women who consented (n=136) were randomly assigned into four groups (with 34 participants per group), and were distributed in one of the four intervention arms- metformin-1gm/day alone, metformin- 1gm/day + VD 4000 IU/d, pioglitazone-30mg/day alone and pioglitazone-30mg/day + VD 4000 IU/d. All the subjects were advised standard lifestyle measures including diet (30-35 Kcal/kg diet with 50-60% carbohydrate, 20-25% protein and 20-25% fat) and exercise (30minutes brisk walk). The women were asked to use barrier contraception during the entire study period. The advice was reinforced at each telephonic or physical visit. The subjects were followed up by telephonic (4 weekly) and physical visits (3, 6 months) for the entire study period (Fig 1).

Glucose tolerance was categorized according to WHO 1999 criteria. A total serum testosterone >48ng/dl was taken as biochemical hyperandrogenism. The assessment of OGTT based insulin sensitivity indices surrogates were calculated as follows:

HOMA-IR = (I0 × G0)/ 22.5, where I= (μU/mL) and G= mmol/dl.

QUICKI = 1/(logI0 + logG0), where I=(μU/mL) and G= mg/dl.

Matsuda= 1000/√G0I0GmeanImean, where I= mIU/L, G= mg/dl.

FGIR=G0/I0

Statistical analysis:Statistical analysis was done using Statistical Package for Social Sciences-22 program (SPSS Inc., Chicago, IL, USA). Data has been shown as mean and standard deviation and data was log transformed wherever necessary. Normality assessment of the parameters was tested with the Kolmogorov–Smirnov test. Kruskal-Wallis H test was applied for comparison in case data was not normally distributed. One-way ANOVA with linear repeated measures and followed by pair wise comparison using Tukey's post hoc test was used for comparison of Gaussian distributed parameters. A p-value<0.05 was considered as statistically significant.

Results

Baseline

Out of a total of 235 women with PCOS, 136 were randomized and data on 119 was available for final statistical analysis (Fig 1).

The mean age of the overall cohort was 21.89±4.99 years with mean BMI of 22.19±3.24kg/m2. Out of the total 119 subjects, 67.34% had irregular menstrual cycles, 24.48% had alopecia, 68.36% had moderate or severe acne, while 81% were vitamin D deficient (serum 25OHD<20mg/ml). The baseline number of cycles per year, FG score, serum total testosterone, fasting glucose and insulin as well as various insulin sensitivity indices were comparable (p>0.05).

Follow Up

Metformin vs. Pioglitazone: In the metformin group, frequency of menstrual cycles improved from 6.92 ± 3.40 at 0 months to 8.76 ± 2.48 (p<0.05) cycles per year while as FG score improved from 10.90±2.26 to 8.24±2.58 at the end of 6 months. Serum total testosterone levels showed a significant decrease, with 6 months of metformin therapy (Table 1). Results from OGTT showed a significant improvement in fasting plasma glucose values and 1 and 2-hour glucose and insulin levels improved significantly over six months as did the insulin sensitivity indices HOMA-IR and Matsuda.With six months of pioglitazone therapy, frequency of menstrual cycles significantly improved from

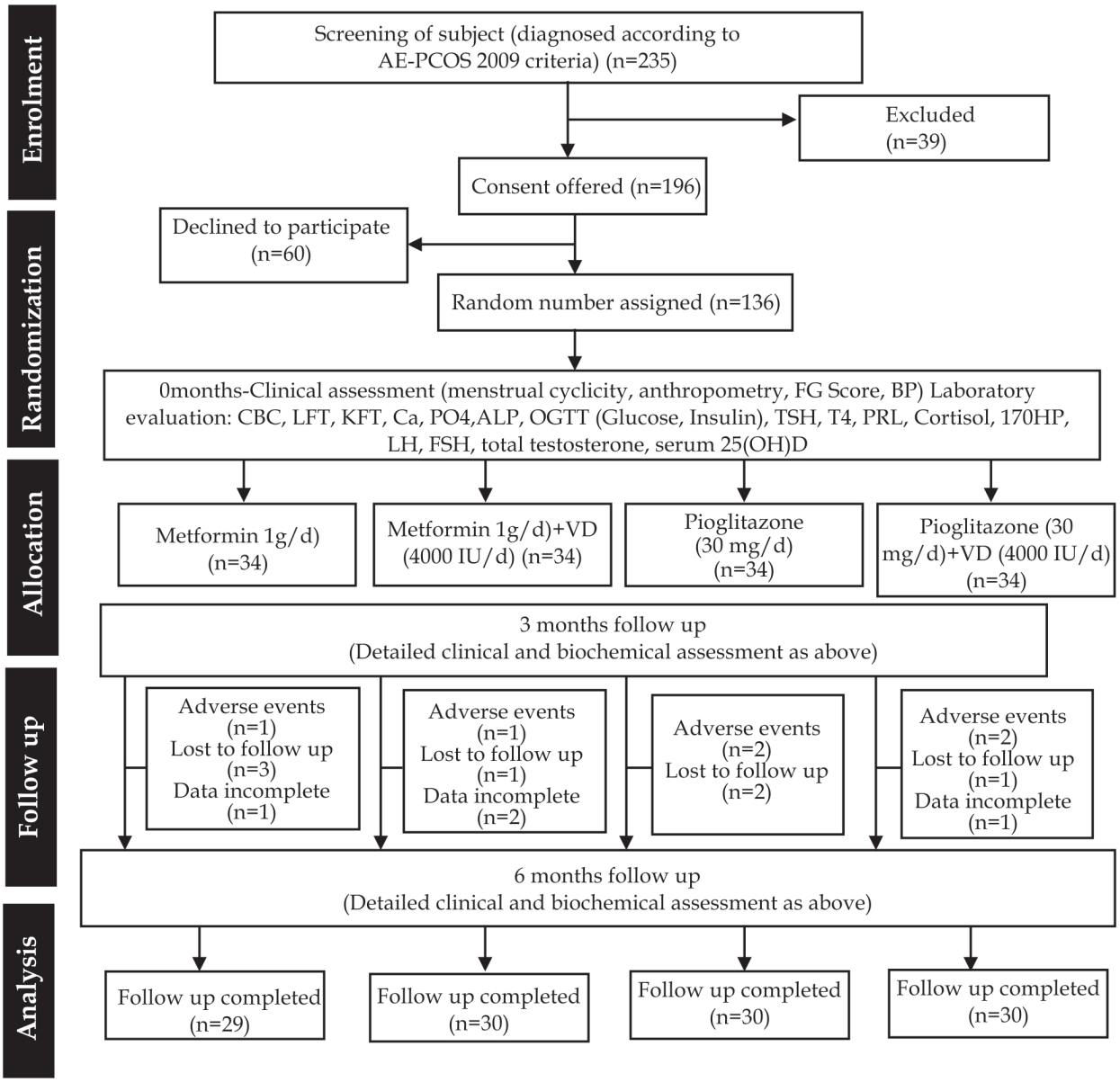

Fig. 1 : Consort chart describing the flow of participants through the randomized trial.

6.89±3.51 to 9.04±3.20 cycles per year. In the pioglitazone arm, mean BMI increased (22.27 ± 3.06kg/m2 to 23.86 ± 3.52kg/m2) with a significant increase in waist circumference ($p<0.05$). The mean FG score significantly decreased (10.83±2.26 to 9.57±1.95) after six months as did the mean serum total testosterone levels (65.62±21.71 to 55.66±26.13ng/dl) ($p<0.05$ vs. metformin group). There was no significant change in SBP, DBP, serum calcium, phosphate and FSH after six months. Similar to metformin group, fasting, 1 and 2-hour plasma glucose and 1-hour insulin levels decreased with a significant improvement in insulin sensitivity indices (HOMA-IR and QUICKI indices) (Table 1).

Metformin with VD

Compared to metformin alone, coadministration of metformin with cholecalciferol increased the frequency of menstrual cycles from 6.97±4.06 at 0 months to 9.42±2.63 per year at the end of 6 months ($p<0.05$ vs. metformin alone). FG score improved from 10.79±2.91 at baseline to 8.00±1.61 after 6 months. Serum total testosterone levels showed a significant decrease with 6 months of metformin+ VD therapy and the effect was significantly superior to metformin alone ($p<0.05$). As expected serum 25(OH)D levels also improved significantly(13.28±8.44 at baseline to 63.33±19.76ng/ml after six months). Total serum testosterone levels improved most significantly

Table 1: Comparing clinical, biochemical, hormonal and insulin sensitivity parameters before and after treatment among women with PCOS receiving metformin vs. pioglitazone.

Parameter Timing of assessment	Metformin (1gm/day) Mean ± SD (N=30)			Pioglitazone (30mg/day) Mean ± SD (N=29)		
	0 month (n=34)	3rd month (n=34)	6th month (n=29)	0 month (n=34)	3rd month (n=34)	6th month (n=30)
No of cycles/year	6.92±3.40	8.38±4.65[a]	8.76±2.48[b]	6.89±3.51	9.39±5.05[a]	9.04±3.20[b,z]
BMI (Kg/m^2)	22.18±3.47	22.35±3.34	22.11±2.95	22.27±3.06	22.86±3.18	23.86±3.52[b,z]
Waist circumference	77.54±8.63	77.41±7.23	76±6.53	77.43±7.79	79.09±7.57	81.09±8.02[b,z]
FerrimanGallwey Score (mFG)	10.90±2.26	10.14±1.82	8.24±2.58[b,c]	10.83±2.26	9.26±2.00	9.57±1.95[b,z]
SBP (mm Hg)	115.71±10.28	113.10±11.01	114.35±12.76	112.30±10.81	116.52±7.14	113.35±9.32
DBP (mm Hg)	78.18±7.76	76.43±8.68	76.09±8.39	74.80±7.96	77.83±4.48	74.78±8.59
Serum LDL cholesterol (mg/dl)	102.33±35.94	102.71±27.38	107.86±27.78	103.74±28.54	100.00±29.11	96.43±23.48
Serum HDL cholesterol (mg/dl)	41.76±12.54	44.95±13.60	44.90±9.42[b]	42.78±8.25	43.26±12.07	44.65±6.99
Serum triglycerides (mg/dl)	124.48±66.21	128.71±53.48[a]	123.29±61.75[b]	123.09±54.18	110.87±47.83	113.57±49.83
Serum total calcium (mg/dl)	9.64±0.39	9.78±0.50	9.57±0.47	9.45±0.49	9.78±0.50	9.66±0.51
Serum phosphate(mg/dl)	3.62±0.59	4.01±0.65	3.88±0.82	3.88±0.94	3.61±0.72	4.05±0.91
Serum ALP (IU/L)	208.67±48.28	171.48±73.50[a]	149.09±67.04[b,c]	204.22±81.67	149.39±67.10[a]	127.09±67.04[b,c]
Serum 25 OHD (ng/ml)	13.09±9.06	10.32±7.90	13.17±7.27	12.70±6.89	9.69±8.93	9.90±5.77
Serum LH (mIU/L)	10.46±6.24	10.15±4.86	10.08±6.78[b]	11.04±10.48	9.85±2.55[a]	8.29±5.96[b,c]
Serum FSH (mIU/L)	6.39±2.78	5.38±1.31	4.93±1.59[b]	6.08±1.53	6.18±1.52	5.90±1.31
Serum testosterone, total (ng/dl)	63.32±38.67	58.25±28.36	51.70±28.38[b,c]	65.62±21.71	60.22±20.78	55.66±26.13[b,c,z]
Plasma glucose-0hr (mg/dl)	85.62±7.83	83.48±6.68	80.71±10.50[b]	84.28±7.61	81.35±8.26	79.09±6.94[b]
Plasma glucose-1hr (mg/dl)	134.65±37.43	131.76±19.34	119.78±42.17[b]	129.22±29.20	115.30±20.12	105.52±24.63
Plasma glucose-2hr (mg/dl)	115.26±24.52	112.76±25.93	108.13±21.21[b]	108.17±23.19	102.22±21.64	99.09±17.42
Plasma insulin-0hr (µIU/ml)	10.98±9.06	9.04±5.34	9.13±5.22	10.89±5.24	8.96±5.64	8.41±3.44
Plasma insulin-1hr (µIU/ml)	79.71±50.41	59.16±28.58	59.21±47.57[b]	77.58±39.37	78.33±37.43	63.07±31.46[b]
Plasma insulin-2hr (µIU/ml)	61.17±41.23	58.20±58.78	37.66±26.62[b,c]	60.90±32.07	57.22±31.39	54.56±34.44
HOMA-IR	2.43±2.11	1.97±1.28	1.91±1.33[b]	2.33±1.22	1.81±1.17	1.65±0.76[b]
QUICKI	0.35±0.03	0.36±0.03	0.36±0.03	0.35±0.03	0.36±0.04	0.36±0.02[b]
Matsuda Index	5.71±2.97	6.85±2.67	7.67±3.39[b]	5.54±2.25	6.54±2.54	7.25±2.66[b]

Results are given as Mean±SD 25 OHD = 25 Hydroxycholecalciferol; ALP= alkaline phosphate Significance, p<0.05: Comparison within the group, a=month 0 vs. month 3; b=month 0 vs. month 6; c=month 3 vs. month 6. Comparison of between the groups, p <0.05; x = month 0; y= month 3 and z= month 6.

in this group from 64.60±23.59ng/dl at baseline to 41.24±12.83 ng/dl after six months of therapy (Table 2). Fasting glucose, 1-hour and 2-hour post OGTT values declined significantly. A significant decrease was also observed in plasma insulin levels after 6 months with improvement in HOMA-IR and Matsuda index at 3 and 6 months of therapy. There was no significant effect on BMI, BP, lipid profile, serum calcium, phosphate and FSH levels (Table 2). Pioglitazone with VD: The combination

Table 2: Comparing clinical, biochemical, hormonal and insulin sensitivity parameters before and after treatment among women with PCOS receiving metformin and cholecalciferol vs. metformin alone.

Parameter Timing of assessment	Metformin (1gm/day) Mean ± SD (N=30)			Metformin (1gm/day) + Vitamin D (4000 IU/d) Mean±SD (N = 30)		
	0 month (n=34)	3rd month (n=34)	6th month (n=29)	0 month (n=34)	3rd month (n=34)	6th month (n=30)
No of cycles/year	6.92±3.40	8.38±4.65[a]	8.76±2.48[b]	6.97±4.06	8.79±3.96[a]	9.42±2.63[b,z]
BMI (Kg/m2)	22.18±3.47	22.35±3.34	21.58±2.98	22.18±3.53	21.78±3.29	21.73±3.79
Waist circumference	77.54±8.63	77.41±7.23	76.23±6.53	78.03±6.15	78.01±5.67	77.43±5.11
FerrimanGallwey Score (mFG)	10.90±2.26	10.14±1.82	8.24±2.58[b,c]	10.79±2.91	8.87±1.98[a]	8.00±1.61[b,c]
SBP (mm Hg)	115.71±10.28	113.10±11.01	113.57±12.76	113.13±6.88	114.37±9.93	114.04±9.08
DBP (mm Hg)	78.81±7.56	76.43±8.68	74.78±8.59	75.00±7.22	75.00±11.12	78.46±7.72
Serum LDL cholesterol (mg/dl)	102.33±35.94	102.71±27.38	107.86±27.78	99.04±31.61	100.21±28.65	102.54±32.57
Serum HDL cholesterol (mg/dl)	41.76±12.54	44.95±13.60	44.90±9.42[b]	41.08±5.71	40.33±7.23	42.46±5.51
Serum triglycerides (mg/dl)	138.48±96.41	128.71±53.48[a]	123.29±61.75[b]	121.33±37.87	112.04±63.09[a]	110.46±33.75
Serum total calcium (mg/dl)	9.64±0.39	9.78±0.50	9.57±0.47	9.80±0.42	9.74±0.57	9.62±0.63
Serum phosphate(mg/dl)	3.62±0.59	4.01±0.65	3.88±0.82	4.06±0.67	3.67±0.69	3.55±0.54
Serum ALP (IU/L)	208.67±48.28	171.48±73.50[a]	149.09±67.04[b,c]	204.88±65.15	200.33±62.99	156.83±65.58[b,c]
Serum 25 OHD (ng/ml)	13.09±9.06	10.32±7.90	13.17±7.27	13.28±8.44	43.94±14.25[a,y]	63.33±19.76[b,c,z]
Serum LH (mIU/L)	10.46±10.24	10.15±4.86	10.08±6.78[b]	10.28±3.26	9.54±4.18[a]	8.26±5.68[b]
Serum FSH (mIU/L)	6.44±2.89	5.38±1.31	5.07±1.55[b]	6.58±1.43	5.68±1.89	5.83±1.92
Serum testosterone, total (ng/dl)	63.32±39.67	58.25±28.36	51.70±28.70[b,c]	64.60±23.59	54.34±23.32[a,y]	41.24±12.83[b,c,z]
Plasma glucose-0hr (mg/dl)	85.62±7.57	83.48±6.68	80.71±10.90[b]	86.00±13.58	83.04±9.75	81.33±16.22[b]
Plasma glucose-1hr (mg/dl)	136.62±37.14	131.76±19.34	120.71±43.97[b]	138.12±32.62	126.50±26.50[a]	111.92±22.77[b]
Plasma glucose-2hr (mg/dl)	115.81±23.19	112.76±25.93	108.19±21.47[b]	118.33±25.50	108.58±54.02	98.75±24.75[b,c,z]
Plasma insulin-0hr (µIU/ml)	10.98±9.06	9.04±5.34	9.13±5.32	10.43±4.40	8.80±3.02[a]	8.55±3.65[b,z]
Plasma insulin-1hr (µIU/ml)	76.51±51.61	59.16±28.58	59.21±47.57[b]	74.37±50.12	58.33±26.65	53.12±32.13[b,z]
Plasma insulin-2hr (µIU/ml)	61.17±41.23	58.20±58.78	37.66±26.62[b,c]	63.65±39.50	55.16±26.51	50.56±27.58[b,z]
HOMA-IR	2.43±2.11	1.97±1.28	1.91±1.33[b]	2.26±1.07	1.53±0.75[a]	1.43±0.76[b]
QUICKI	0.35±0.03	0.36±0.03	0.36±0.03	0.35±0.02	0.37±0.03[a]	0.37±0.04[b]
Matsuda Index	5.71±2.97	6.85±2.67	7.67±3.39[b]	4.55±1.45	6.18±1.99[a]	7.61±3.65[b,c]

Results are given as Mean±SD 25 OHD = 25 Hydroxycholecalciferol; ALP= alkaline phosphate, significance, $p<0.05$: Comparison within the group, a=month 0 vs. month 3; b=month 0 vs. month 6; c=month 3 vs. month 6. Comparison of between the groups, $p<0.05$; x = month 0; y= month 3 and z= month 6.

Table 3: Comparing clinical, biochemical, hormonal and insulin sensitivity parameters before and after treatment among women with PCOS receiving pioglitazone and cholecalciferol vs. pioglitazone alone.

Parameter	Pioglitazone (30mg/day) Mean ± SD (N=29)6			Pioglitazone (30 mg/day) + Vitamin D (4000 IU/d) Mean ± SD (N = 30)3		
	0 month (n=34)	3rd month (n=34)	6th month (n=30)	0 month (n=34)	3rd month (n=34)	6th month (n=30)
No of cycles/year	6.89±3.51	9.39±5.05[a]	9.04±3.20[b]	7.01±3.33	8.24±2.27[a]	10.24±2.47[b]
BMI(Kg/m2)	22.27±3.06	22.86±3.18	23.86±3.52[b]	22.12±2.90	22.80±3.15	23.22±3.05
Waist circumference(cm)	77.43±7.79	79.09±7.57	81.09±8.02[b]	78.84± 6.56	79.04±8.14	78.96±6.74
FerrimanGallwey score (mFG)	10.83±2.26	9.66±2.00	9.57±1.95[b]	10.76±2.91	8.85±1.41[a]	8.48±1.26[b,z]
SBP (mm Hg)	111.96±11.15	116.52±7.14	113.35±9.32	114.40±11.12	110.60±9.60[y]	111.40±12.35
DBP (mm Hg)	74.57±8.24	77.83±4.48	74.78±8.59	74.40±7.41	72.80±8.32	75.40±7.89
Serum LDL Cholesterol (mg/dl)	103.74±28.54	100.00±29.11	96.43±23.48	97.80±27.47	104.12±41.50	105.88±38.96
Serum HDL Cholesterol (mg/dl)	42.78±8.25	47.26±12.07	44.65±6.99	37.12±8.84	37.84±9.91	42.88±10.00[b]
Triglycerides (mg/dl)	123.09±54.18	110.87±47.83	113.57±49.83	115.68±42.07	118.72±55.47	115.88±52.48
Total Serum calcium (mg/dl)	9.45±0.49	9.78±0.50	9.66±0.51	9.60±0.44	9.59±0.75	9.46±0.30
Serum phosphate(mg/dl)	3.88±0.94	3.61±0.72	4.05±0.91	4.00±1.11	3.55±0.53	3.63±0.43
Serum ALP (IU/L)	204.22±81.67	149.39±67.10[a]	127.09±67.04[b]	209.52±54.54	150.08±77.90[y]	126.12±53.85[b,z]
Serum LH (mIU/L)	11.04±10.48	9.85±2.55[a]	8.29±5.96[b,c]	10.46±6.16	9.63±4.94[y]	8.46±6.41[b,c]
Serum FSH(mIU/L)	6.08±2.01	6.18±1.52	5.90±1.31	6.95±1.56	6.23±1.62	6.14±2.25
Serum testosterone, total (ng/dl)	65.62±21.71	60.22±20.78	55.66±26.13[b]	67.09±30.08	58.90±31.04[a,y]	51.55±14.34[b,c,z]
Serum 25 OHD (ng/ml)	12.70±6..89	9.69±8.93	9.90±5.77	11.17±7.46	47.06±12.54[a,y]	57.69±14.43[b,c,z]
Plasma glucose-0hr (mg/dl)	84.65±7.74	81.35±8.26	79.09±6.94[b]	85.84±11.28	80.80±9.74[y]	70.28±9.64[b,c,z]
Plasma glucose-1hr (mg/dl)	129.22±29.20	119.30±20.12	115.52±24.63	129.32±34.32	123.36±20.96[y]	119.93±21.83[b,c]
Plasma glucose-2hr (mg/dl)	108.17±23.19	102.22±21.64	99.09±17.42	112.60±31.88	105.32±22.38	95.16±32.85[b,c]
Plasma insulin-0hr (µIU/ml)	10.99±5.43	8.96±5.64	8.41±3.44	10.77±6.31	9.54±17.84[a]	7.66±4.77[b,c,z]
Plasma insulin-1hr (µIU/ml)	77.58±39.37	78.33±37.43	63.07±31.46[b]	69.44±30.95	54.36±29.01[a,y]	44.40±20.28[b,c,z]
Plasma insulin-2hr (µIU/ml)	60.90±32.07	57.22±31.39	54.56±34.44	51.02±56.32	49.20±27.90	32.39±20.28[b,z]
HOMA-IR	2.33±1.22	1.81±1.17	1.65±0.76[b]	2.44±1.43	1.39±1.46[a]	1.22±0.95[b]
QUICKI	0.35±0.03	0.36±0.04	0.36±0.02[b]	0.34±0.03	0.36±0.04[a]	0.36±0.03[b]
Matsuda Index	5.54±2.25	6.54±2.54	7.25±2.66	5.28±3.13	7.64±3.17[a]	8.72±3.94[b,z]

Results are given as Mean±SD 25 OHD = 25 Hydroxycholecalciferol; ALP= alkaline phosphate. Significance, $p<0.05$: Comparison within the group, a=month 0 vs. month 3; b=month 0 vs. month 6; c=month 3 vs. month 6. Comparison of between the groups, $p<0.05$; x = month 0; y= month 3 and z= month 6

of pioglitazone with cholecalciferol significantly improved the menstrual cyclicity from a baseline value of 7.01±3.33 to 10.24±2.47 cycles per year after six months. FG score significantly decreased from 10.76±2.91 to 8.48±1.26 after six months. The mean BMI marginally increased with no effect on BP, total serum calcium, triglycerides. Total serum testosterone levels improved most significantly

in this group from 67.09±30.08ng/dl at baseline to 51.55±14.34ng/dl after six months of therapy (Table 3). Contrary to pioglitazone alone, the decrease was significant after three months of pioglitazone+VDcoadministration which also showed a superior effect in decreasing fasting plasma glucose and 0, 1 and 2hour post OGTT insulin levels ($p<0.05$ vs. pioglitazone group).

Metformin with VD vs. Pioglitazone with VD

Compared to pioglitazone+VD, metformin+VD was effective in improving mean FG score (10.79±2.91 to 8.00±1.61 vs. 10.76±2.91 to 8.48±1.26), reducing serum total testosterone (64.60±23.59 to 41.24±12.83ng/dl vs. 67.09±30.08 to 51.55±14.34ng/dl) after six months of therapy. Contrary to pioglitazone+ VD group, serum total testosterone was significantly reduced after only 3 months of metformin+VDcoadministration. However, there was a significantly superior effect on fasting plasma glucose (85.84±11.28 to 70.28±9.64 vs. 86.00±13.58 to 81.33±16.22mg/dl) as well as insulin sensitivity indices such as HOMA-IR and Matsuda indices on pioglitazone+VDcoadministration ($p<0.05$ vs. metformin+VD group) (Supplementary Table 1).

Adverse Events

Very few adverse events were recorded during the trial. In the metformin+ VD group, one subject complained of diarrhoea while as two subjects in the pioglitazone alone group complained of puffy face. In the pioglitazone+ VD group two adverse events were recorded from two subjects, one complaining of abdominal pain while other had deranged LFT values, leading to withdrawal of therapy.

Discussion

In the present study, we evaluated the efficacy of insulin sensitizers- metformin and pioglitazone alone and in combination with high dose VD, on various clinical, biochemical and hormonal parameters among women with PCOS. While metformin was beneficial in improving menstrual cycles, features of hyperandrogenism such as FG score, serum total testosterone, pioglitazone proved to be better in improving insulin sensitivity parameters, despite moderate gain in body weight. Coadministration of high dose cholecalciferol with metformin or pioglitazone marginally improved efficacy of either drug with effect being more pronounced in pioglitazone + VD group.

Both metformin and pioglitazone have been shown to reduce hyperandrogenemia, restore menstrual cycles and improve ovulation in women with PCOS. In our previously published data comparing the efficacy of spironolactone (50 mg/d) with metformin (1000 mg/d) after random allocation among 82 young women with PCOS, metformin was significantly more effective in improving insulin sensitivity while as spironolactone appeared superior in treatment of hirsutism, frequency of menstrual cycles and hormonal derangements.[9] Additionally, we have shown that combination of low-dose spironolactone with metformin was superior to either drug alone in management of clinical parameters in 169 PCOS subjects, randomized into 3 groups receiving metformin (1000 mg/d), low-dose spironolactone (50 mg/d), or a combination of both drugs for a period of 6 months.[23] TZD efficacy can be expected to be similar to metformin, though a small study demonstrated pioglitazone to be more effective in improving menstrual irregularities and hirsutism score after six months of treatment.[13] Similar to earlier reports, the mean FG-score and menstrual cyclicity improved with six months of metformin and pioglitazone therapy[11], pioglitazone being more effective in ameliorating insulin resistance as compared to metformin. With pioglitazone therapy, anthropometric parameters including mean body weight, BMI and waist circumference increased post-treatment as reported previously.[24] This weight gain however, did not seem to obviate the clinical benefits.

VDD in PCOS has been linked to several non-skeletal effects which include association with obesity, NASH, IR, DM, CVDetc.[14,15,18] In addition, VDD may modulate the response to various treatment modalities in PCOS patients. In the present study, co-administration of cholecalciferol to either metformin or pioglitazone seemed to improve the clinical and biochemical parameters in terms of improvement in the frequency of menstrual cycles, FG-score, serum testosterone as well as insulin sensitivity indices compared to either drug alone.

There are no previous studies assessing the effect of pioglitazone combined with VD in PCOS subjects. A few available studies evaluating the efficacy of metformin and VD co-administration did show favorable results. A three month study on 60 women with PCOS administering calcium–vitamin D along with metformin was effective in restoring menstrual cycles[26], similar to the observations by Firouzabadi et al in 100 infertile women.[27] Consistent with our findings, administration of high dose cholecalciferol (4000IU) in women with PCOS for 12 weeks led to a significant improvement in serum

testosterone and SHBG levels in comparison to the low dose and placebo group.[22]

The results support our observations as we used the same dose. The findings are, however, in contrast to a recent meta-analysis concluding that VD supplementation had no role in mitigation of metabolic or hormonal features in women with PCOS.[16] In the present study, twelve weeks of VD supplementation improved HOMA-IR and QUICKI among PCOS women consistent with the results published earlier.[28] Contrary to our findings, a meta-analysis assessing the impact of VD supplementation in PCOS women found no difference between hormonal and insulin sensitivity parameters in the placebo and VD group.[29]

A recent study on 251 patients with T2D nephropathy showed that VD in combination with pioglitazone was effective in improving bone metabolism than either VD or pioglitazone alone. While it is still a point of debate, studies have found a correlation between low serum concentrations of 25OHD and metabolic derangements in women with PCOS.[30] The results from our study show a superior effect of the pioglitazone-VD combination on insulin sensitivity as compared to metformin-VD group or with pioglitazone or metformin alone. [31] Although we have not assessed inflammatory markers or visceral fat, pioglitazone may work by converting insulin resistant fat to insulin sensitive fat.[32]

Further, addition of VD may affect glucose homeostasis via certain direct mechanisms such as binding of circulating 25OHD to VD receptor on beta cell surface as well as indirectly via regulation of calcium influx in insulin responsive tissues.[33] Pioglitazone reduces IR in liver and peripheral tissues by selectively stimulating Peroxisome proliferator-activated receptor gamma (PPAR-γ), a type II nuclear receptor that activates downstream genes regulating glucose metabolism. High dose VD supplementation has also been shown to upregulate expression of PPAR-γ, thus may synergistically improve insulin resistance.[34]

Although limited data comparing metformin with pioglitazone has been published, this is the first study, to the best of our knowledge, comparing the effect of VD co-supplementation with metformin or pioglitazone among women with PCOS. Limitations of our study include low sample size and lack of a control VD arm. Also, the cholecalciferol supplementation should have been started earlier to make the subjects VD replete.

Conclusion

Metformin was effective in improving features of hyperandrogenism while as pioglitazone scored over metformin in improving insulin sensitivity parameters, despite moderate increase in BMI. Coadministration of high dosage VD was beneficial in improving both clinical and biochemical outcomes. This is the first open labelled randomized study assessing the effect of VD supplementation in combination with metformin and pioglitazone. The results pave way for further studies with large sample size, to assess the combinatorial effect of metformin and pioglitazone in vitamin D replete subjects with longer follow up.

List of Abbreviations

PCOS	Polycystic Ovary Syndrome
VDD	Vitamin D Deficiency
AE-PCOS	AE- Polycystic Ovary Syndrome
25OHD	25-Hydroxy vitamin D
FG	FerrimanGallwey
OGTT	Oral Glucose Tolerance Test
MS	Metabolic Syndrome
IR	Insulin Resistance
AGT	Abnormal Glucose Tolerance
NASH	Non-alcoholic Steato hepatitis
IRIS	Insulin Resistance Intervention after Stroke
NCAH	Nonclassical congenital adrenal hyperplasia
BMI	Body Mass Index
ELISA	Enzyme Linked Immunosorbent Assay
WHO	World Health Organization
FGIR	Fasting Glucose to Insulin Ratio
TZD	Thiazolidinediones
DM	Diabetes Mellitus
CVD	Cardio vascular Diseases

Declarations

Compliance with Ethical Standards: An informed written consent was taken from all the participating subjects. The study was done in accordance with the Helsinki declaration 1975 and was approved by Institute Ethics Committee, All India Institute of Medical Sciences, (AIIMS), New Delhi.

Data availability: The datasets used and/or analysed during current study are available from the corresponding author on reasonable request.

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Author Contributions: Conception and design of the study (MAG, MAZ, ZAS), data acquisition (AR, IAW, SH, RK), analysis and interpretation of data (MAG, TS), drafting the article (MAG, TS) and final submission of the article (MAG).

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