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Original research article

A prospective comparative study of role of Vitamin D supplementation in women with PCOS

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Abstract

The present study is a hospital based prospective study conducted in the department of Obstetrics & Gynaecology. A total of 100 women diagnosed with PCOS with Vitamin D deficiency fulfilling the inclusion criteria were included in to the study and are sub divided in to two groups A & B with 50 women in each group. Group A: Women were given treatment with Metformin & Calcium 1000mg daily along with Vitamin D3 60K IU weekly for 6weeks followed by monthly for 4 months. Group B: Women were given treatment with Metformin 1000mg daily only. Detailed history regarding chief complaints, menstrual cycles, hyperandrogenic symptoms like hirsutism & acne, history of features of PCOS in the family was taken. The cases in both the groups are matched well with regards to mean age, BMI, waist hip ratio, percentage of acne & hirsutism positive women, menstrual irregularities, mean ovarian volume, mean serum total testosterone & mean serum fasting insulin before treatment. Follow up was done during the treatment and at the end of the 6 months again women were examined clinically and investigated to access any improvement in the various features of PCOS we considered. In group A with Metformin, Vitamin-D & Calcium supplementation after treatment 78% had regular menstrual cycles (p=0.0001), where as in group B with Metformin supplementation only 56% showed regularization of cycles. Oncomparing between the two groups it was found that addition of Vitamin D & Calcium to Metformin regularize the menstraul cycles significantly with p value 0.031. In relation to mean ovarian volume, after 6months of treatment there was a significant reduction by 30% (p <0.0001) in group A i.e. Vitamin D, Calcium & Metformin supplemented women. Where as in group B i.e. Metformin supplemented group there was only a 13% reduction in mean ovarian volume after 6 months of treatment. On comparing the two groups, the women in group A showed a significant reduction in mean ovarian volume when compared to other group, which signifies the decrease in stromal thickness, androgen production favouring the maturation of antral follicles to dominant follicle & its ovulation. The mean serum total testosterone in group A showed significant reduction by 22% in mean value after 6 months of supplementation with Vitamin D, Calcium & Metformin (p value <0.01). Where as in group B with Metformin alone there was only 13% reduction in mean serum total testosterone (p value 0.01). On comparing the two groups showed a significant difference(p =0.003) in reduction in mean total testosterone after 6 months of treatment in women received Vitamin D, Calcium & Metformin when compared to women received Metformin alone.

Keywords: Vitamin D, Calcium & Metformin, hirsutism, testosterone, antral follicles

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder of women of reproductive age affecting about 6-15% [1-4] of all women. A complex interaction of various factors contribute to the pathogenesis of PCOS. The most widely accepted theory proposes that PCOS is a self-perpetuating cycle of hormonal events with arrested follicular development, increased androgen concentration resulting in polycystic ovaries [5].

PCOS is charecterized by increased ovarian & adrenal androgen secretion, hyperandrogenic symptoms like hirsutism, acne, alopecia, menstrual irregularities & polycystic ovaries with increased stromal thickness & ovarian volume [6]. PCOS is associated with multiple risk factors such as insulin resistance, central obesity, subclinical atherosclerosis, hypertension, impaired glucose tolerance, type 2 DM, metabolic syndrome, dyslipidaemia, infertility, endometrial hyperplasia, endometrial & ovarian cancers

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[3]. Early detection and treatment of the disease is absolutely important.
Diagnosing PCOS is not easy as signs and symptoms are heterogeneous and vary from time to time. Diagnostic criteria have been summarized by the European Society for Human Reproduction (ESHRE) and the American Society for Reproductive Medicine (ASRM) in 2003, and denominated as 'Rotterdam Criteria' [7]. Recently in 2006 Androgen excess society developed a criteria making androgen excess as a necessary component for diagnosing PCOS [8].
The optimal management of PCOS is uncertain & treatment focuses on ameoliration of clinical features [2]. Metformin administered to women with PCOS increases the frequency of ovulation, menstrual regularization, improvement in severity of hyper androgenic symptoms & decreases the insulin resistance by possibily decreasing the androgen levels [2, 3].
Vitamin D deficiency has been proposed as the possible missing link between IR and PCOS. This assumption is supported by the finding that the active vitamin D & its receptor (VDR) complex regulates over 300 genes, including genes that are important for glucose and lipid metabolism as well as blood pressure regulation [9]. Association between Vitamin D status and metabolic and hormonal disorders in women suffering from PCOS is scarce, many studies have concluded that Vitamin D supplementation has increased insulin sensitivity & improvement of PCOS symptoms [5-10].
Considering that proper interventions could improve outcomes of PCOS & its related long term complications, we aimed in our study to evaluate & compare the effect of vitamin D & calcium supplementation in addition to Metformin to Metformin alone in PCOS women.

Patients and Methods
Data Sources
This is a prospective study done in the Department of Obstetrics and Gynaecology.
This study was designed to compare the efficacy of vitamin D supplementation over Metformin in the management of PCOS with Vitamin D deficiency.

Source of the Study
Women attending to Gynaecological Department with complaints of menstrual irregularities/hirsutism/ acne are recruited.

Type of Study: Prospective randomised study.

Sample Size: 100 subjects with 50 in each Group A & Group B.

Inclusion Criteria
- Women diagnosed PCOS between ages 15-35 yrs are included in the study.

Exclusion Criteria
- Patient not giving consent for the study.
- Patients who have conceived in period of our study.
- Patients with lost follow up.
- Patients taking infertility treatment.
- Patients who underwent any surgical procedures during study.
- Patients with cardiac/renal/hepatic/thyroid disorders & hyperprolactinemia.

Materials and Methods
- Women attending OPD diagnosed as PCOS are recruited into the study & Vitamin D levels are estimated. 100 women with diagnosed PCOS and Vitamin D deficiency are recruited in to the study.
- Women who have features suggestive of PCOS, but who do not fulfill the criteria were excluded from the study.
- Consent was taken from the selected woman after explaining the study design.
- The detailed history (demography, complaints, menstrual, medical & surgical) was taken & clinical examination (complete general physical examination, systemic) was done.
- Investigations like sr. Total testosterone, sr. Fasting insulin, Transabdominal ultra sound for ovarian morphology and volume was done.
- Selected women are randomly divided into two groups.
- The group A was supplemented with Metformin 1000mg daily along with Vit D3 60k IU weekly for 6 weeks followed by monthly for 4months & Calcium 1000mg daily.
- The group B was supplemented only with Metformin 1000mg daily for 6months.
- The cases were followed & response to treatment was assessed (menstrual cycles, hyperandrogenic features, insulin resistance, ovarian morphology & volume) after 6 months.
- The results were compared between the two groups after statistical analysis.
- The data was tabulated and analyzed. Categorical data was analyzed by chi square test and student T

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test of proportion.

- Multivariate analysis was performed to establish the relationship between Vitamin D3 & Calcium supplementation and outcome along with Metformin in diagnosed Vit D3 deficient PCOS women.

Anthropometric Measurements

- Parameters such as height, weight & BMI were recorded.
- Weight was measured using a beam balance, to the nearest 0.1 kg and height to the nearest centimetre, using a tape stuck to the wall.
- The BMI was calculated as the weight in kilograms divided by the square of height in meters.
- Waist circumference was measured in a standing position at the narrowest level between the lower costal margin and the iliac crest. The hip circumference was measured at the widest level in a standing position over the buttocks with a soft tape while the women breathed normally. W-H ratio was calculated & >0.81 was considered abnormal.
- The degree of hirsutism was assessed using the modified Ferriman and Gallwey scoring system. This system grades terminal hair growth on a scale from 0 (no terminal hairs) to 4(extensive terminal hair growth) on 9 anatomical sites (upper lip, chin, chest, upper back, lower back, upper abdomen, lower abdomen, arm, and thigh) and sum of nine areas generate an overall hirsutism score.
- Total score <6-normal, 6-8 mild, 8-15 moderate, >15 overt hirsutism.
- Menstrual cycles, acne are noted from the patient history & clinical examination.
- Ovarian parameters are obtained by Tran's abdominal ultrasound using Philips HD 11 machine.

Vitamin D: Serum 25-OH vitamin D was estimated by high performance liquid chromatography (HPLC) with commercial column and reagents from RECIPE (Germany) and Younglin HPLC (Korea).

Procedure

1. Switch on the pump with flow rate 0.2 ml/min and increase the flow each 2 min interval time till reach the flow rate 1 ml/min.
2. Set the column heater to 40 ºC.
3. Set the UV detector to 264 nm wavelength.
4. Open the method and check all the HPLC parameter settings HPLC parameter setting to check was as follows:

- Flow rate 1 ml/min.
- Column heater 40 ºC.
- Injection volume 50 µl.
- Injection interval 12 min.
- Stop run time 12 min.
- UV detector 264 nm wavelength.

5. Allowed the mobile phase to continue circulating for further 15 to 20 min.
6. Check the baseline into the computer from HPLC software. If the base line was stable then prepare the calibrator/control/patient samples as per the instruction manual, 50 µl each of them one by one.
7. Once the chromatograms are ready, integrate the peaks if required, then calculate the concentration of patient sample with given formula.
8. Concentration of Analyte = Area of sample X Conc. of analyte of calibrator Area analyte of calibrator X recovery.
9. After running all the samples switch off all the modules of HPLC system if not in use.

Serum Testosterone & Insulin

Estimation of Fasting insulin, Testosterone levels were done in serum by chemiluminescence immunoassay (CLIA) using Beckman Coulter Access 2 fully automated analyzer. A common procedure was described for the above hormones.

Principle: The essential reagents required for an immunoenzymatic assay include high affinity and specific antibodies (enzyme conjugated and immobilized), with different and distinct epitope recognition in excess, and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added anti biotinylated monoclonal antibody. Upon mixing mononclonalbiotinylated antibody, the enzyme labeled antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies, without competition or steric-hinderance, to form a soluble sandwich complex. Simultaneously, the complex was deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody.

Once the equilibrium was attained, the antibody bound fraction was separated from unbound antigen by decantation/aspiration. The enzyme activity in the antibody bound fraction was directly proportional to

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the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of a known can be ascertained.

Statistical Analysis

For categorical variables, the data values are represented as number and percentages. To test the association between the groups, chi-square test was used. For continuous variables, the values are represented as mean and standard deviation. To test the mean difference between two groups, student's t-test (Independent sample t-test/paired sample t-test) was used. To test the correlation between the groups, Pearson's correlation was used. All p values are having less than 0.05 are considered as statistical significant.

Results

The mean age of group A & B was 24.24 ± 3.34 & 23.4 ± 4.12 respectively, which is similar. The majority of women with PCOS 72% in group A & 70% in group B are with severe Vitamin D deficiency with value < 15ng/ dl in both the groups. The mean Vitamin D levels are 12.32 ± 4.37 & 13.14 ± 4.34 in group A & B respectively showing high prevalence of Vitamin D deficiency in PCOS women.

Most of the women in both groups are overweight with BMI between 26-30 kg/m^2. Majority of women in group A (50%) & in group B (42%) are in this range. The mean BMI in group A & B are 26.63 ± 3.48 & 25.95 ± 3.69 respectively, which is comparable.

The majority of cases in the both groups A (50%) & B (40%) W-H ratio (0.81-0.86) is above the normal range with elevated risk. 38% in both groups are in high risk with W-H ratio >0.87.The mean W-H ratio in group A 0.85 ± 0.38 & in group B 0.84 ± O.44 which is similar in two groups.

At the end of treatment the women with W-H ratio > 0.87 has decreased to 12% in group A & 16% in group B from 38% in both groups. The mean ratio in group A 0.82 ± 0.36 & in group B 0.82 ± 0.43, showing no significant differences.

Table 1: Menstrual abnormalities Before Treatment-Group A v/s B

Menstrual abnormalities	Group-A		Group-B		P-Value
	(n = 50)	(%)	(n = 50)	(%)	
Amenorrhea	12	24	5	10	0.114
Irregular	17	34	20	40	
Oligo	19	38	25	50	
Regular	2	4	0	0	

Majority of women in group A were with oligomenorrhea (38%) & irregular cycles (34%) where as in group B 50% were with oligomenorrhea & 40% were with irregular cycles, which were similar.

At the end of the treatment 82% of women in group A were with regular cycles when compared to only 46% in group B, with significant p value 0.031. Thus there was a significant improvement in the regularization of menstrual cycles in group A receiving Metformin & Vitamin D & Calcium when compared to group B receiving only Metformin.

Table 2: Acne Before & after Treatment-Group A v/s B

Acne	Group-A		Group-B		P-Value	Group-A		Group-B		P-Value
	(n = 50)	(%)	(n = 50)	(%)		(n = 50)	(%)	(n = 50)	(%)	
Present	20	40	22	44	0.685	11	22	11	22	1.000
Absent	30	60	28	46		39	78	39	78	

Acne before treatment was noted in 40% women in group A & 44% in group B which is same in the two groups. At the end of the treatment, acne was noted in only 22% women in both groups. It shows there is reduction in percentage of acne positive cases in both groups but no significant differences between the groups.

Table 3: Hirsutism before Treatment-Group A v/s B

Hirsutism Score	Group-A		Group-B		P-Value
	(n = 50)	(%)	(n = 50)	(%)	
7-12	10	40	14	58	0.962
13-15	9	36	5	21	
> 15	6	24	5	21	
Mean	**13.16 ± 2.89**		**13.21 ± 4.17**		

In group A 36% & in group B 21% were mild hirsuits with score13-15. 24% in group A & 21% in group

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B was severe hirsuits with score >15.The mean score in group A 13.16 ± 2.89 & in group B 13.21 ± 4.17 which was similar in two groups.

Table 4: Ovarian Volume before Treatment-Group A v/s B

Volume in Cm^3	Group-A		Group-B		P- Value
	(n = 50)	(%)	(n = 50)	(%)	
<7	12	24	13	26	0.765
7-10	31	62	30	60	
> 10	7	14	7	14	
Mean	**8.62 ± 1.67**		**8.52 ± 1.65**		

Majority (62%) in group A & 60% in group B were with ovarian volume between 7-10 cm^3. The mean volume in group A was 8.62 ± 1.67 & in group B was 8.52 ± 1.65 which is similar.

Table 5: Serum Testosterone before Treatment-Group A v/s B

ng/ml	Group-A		Group-B		P-Value
	(n = 50)	(%)	(n = 50)	(%)	
0.2-1	22	44	19	38	0.235
1.1-1.5	28	56	29	58	
1.6-2	0	0	2	4	
Mean	**1.08 ± 0.19**		**1.13 ± 0.22**		

At the end of the treatment women with sr.Fasting Insulin <15 mIU/ml were 40% in group A & 34% in group B. 52% in group A & 56% in group B were with insulin between 16-25 mIU/ml. The mean value in group A 16.01 ± 4.89 & group B 17.03 ± 5.77 showed no statistical significance.

Discussion

PCOS is one of the most common cause of menstrual irregularity and androgen excess in woman, characterized by hyperandrogenism, chronic anovulation, infertility, irregular menstruation & hirsutism affecting 15-20% of women.

An increased physical activity is recommended although this often present limitations. Bariatric surgery has been advocated as a weight loss in the morbidly obese who failed to lose weight with diet & exercise. Insulin sensitizing agents commonly used are Metformin a Biguanide is associated with increased menstrual cyclicity, improved ovulation & a reduction in circulating androgen levels. Its primary clinical action is to inhibit hepatic glucose production & by increased glucose utilization. Other drugs like Glitazones are also tried but there are limited studies on its safety and efficacy.

These are supported by studies showing association between low serum 25OHD levels and increased C-reactive protein levels [9].

PCOS is most common endocrine disorder of reproductive age group women. Incidence is high in the women between 18-30 years. Sometimes PCOS presents in post pubertal period to late reproductive period.

In all the above mentioned studies, the mean age of distribution in the group A (Vitamin D, Metformin & Calcium) was found to be between 25.8-28.7 yrs. In the present study Vitamin deficient women are 99%, among them 56% are mild deficit with vitamin D levels between 11-20 ng/ml, 34% are moderate deficit with 5-9 ng/ml & 9% are severe deficit with vitamin D levels< 5 ng/ml.

There are no documented studies till date available showing the positive effect of Vitamin D, Calcium, & Metformin supplementation decreasing W-H ratio after treatment in PCOS women. Present study showed a reduction in mean W-H ratio by 3.6% after treatment for 6 months.

On comparing the treatment efficacies in both groups (A & B), the mean waist hip ratio was reduced by 3.6% in group A & 2.5% in group B showing no differences between the two groups.

Present study showed a reduction of 39% in hirsutism positive women after treatment for 6months which is in par with others.

While comparing the treatment efficacies of group A & B, 78% in group A & 56% in group B showed a significant difference in improvement of regular menstrual cycles after 6 months of treatment showing addition of Vitamin D & Calcium to Metformin is more efficacious in regularization of cycles in PCOS women.

There are no reference studies available till date comparing the ovarian volume before and after treatment with Metformin, Vitamin D, Calcium.

The present study showed a significant improvement by 30% in reduction of ovarian volume after treatment, which indirectly signifies the more chances for maturation of follicles.

While Comparing the efficacy between the two groups, group A showed 30% reduction & group B showed 13% reduction in ovarian volume showing group A is more potent in reducing ovarian volume when compared to group B with p value

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<0.0001. This shows addition of Vitamin D & Calcium to Metformin improves the efficacy of treatment in reducing the ovarian volume & increases the rate of follicle maturation.
Vitamin D by decreasing IR promotes increased production of SHBG & decreases the androgen production from ovaries leading to low levels of circulating free androgens, thereby decreasing the clinical & biochemical hyperandrogenemia.
On comparing the potency of outcome between the two groups, group A with 22% & group B with 13% reduction in mean serum total testosterone showed significant differences with p value 0.003. The women in group A who received combination of Vitamin D, Calcium & Metformin showed a significant reduction in mean serum total testosterone when compared to women in group B who received Metformin alone proving the effectiveness of addition of Vitamin D & Calcium to Metformin.
Metformin an insulin sensitizing agent decreases the blood glucose levels by decreasing production & increasing the utilization there by decreasing the need for high insulin levels for glucose homeostasis without changing the insulin pharmacokinetics. In the present study, while comparing the efficacy between two groups, there is 18.4% in group A & 13% in group B reduction in the mean serum Fasting insulin showing there is no significant differences in outcome i.e. reducing the serum fasting insulin on addition of Vitamin D & Calcium to Metformin.

Conclusion

PCOS is the most common endocrine problem affecting the women from menarche to perimenopausal age. Vitamin D deficiency is very common in PCOS. A variety of treatment strategies have been developed based on the patient symptomology &need. Insulin sensitizers specially Metformin has proven efficacy in treatment of PCOS since long time. Vitamin D & Calcium supplementation in addition to Metformin has shown a potential therapeutic benefits in ameliorating the hormonal milieu and a variety of PCOS related symptoms mainly menstrual regularity, ovualation & some features of hyperandrogenism.
Thus Vitamin D & Calcium is a safe drug with less side effects& thus recommended for these group of women especially those with Vitamin D deficiency.

References

1. Garg G, Kachhawa G, Ramot R, Khadgawat R, Tandon N, Sreenivas V, *et al*. Effect of vitamin D supplementation on insulin kinetics and cardiovascular risk factors in polycystic ovarian syndrome: a pilot study. Endocrine connections. 2015;4(2):108-16.
2. Rashidi B, Haghollahi F, Shariat M, Zayerii F. The effects of calcium-vitamin D and metformin on polycystic ovary syndrome: a pilot study. Taiwanese Journal of Obstetrics and Gynecology. 2009;48(2):142-7.
3. Tehrani HG, Mostajeran F, Shahsavari S. The effect of calcium and vitamin D supplementation on menstrual cycle, body mass index and hyperandrogenism state of women with poly cystic ovarian syndrome. J Res Med Sci. 2014;19:875-80.
4. Diamanti-Kandarakis E, Kouli CR, Bergiele AT, Filandra FA, Tsianateli TC, Spina GG, *et al*. A survey of the polycystic ovary syndrome in the Greek island of Lesbos: hormonal and metabolic profile. The Journal of Clinical Endocrinology & Metabolism. 1999;84(11):4006-11.
5. Thys-Jacobs S, Donovan D, Papadopoulos A, Sarrel P, Bilezikian JP. Vitamin D and calcium dysregulation in the polycystic ovarian syndrome. Steroids. 1999;64(6):430-5.
6. Wehr E, Trummer O, Giuliani A, Gruber HJ, Pieber TR, Obermayer-Pietsch B. Vitamin D-associated polymorphisms are related to insulin resistance and vitamin D deficiency in polycystic ovary syndrome. European journal of endocrinology. 2011;164(5):741-9.
7. Rotterdam E, ASRM-Sponsored P. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertility and sterility. 2004;81(1):19.
8. Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Futterweit W, *et al*. Criteria for defining polycystic ovary syndrome as a predominantly hyperandrogenic syndrome: an androgen excess society guideline. The Journal of Clinical Endocrinology & Metabolism. 2006;91(11):4237-45.
9. Krul-Poel Y, Snackey C, Louwers Y, Lips P, Lambalk CB, Laven J, *et al*. The role of vitamin D in metabolic disturbances in polycystic ovary syndrome: a systematic review. European Journal of Endocrinology. 2013;169(6):853-65.
10. Kopera D, Wehr E, Obermayer-Pietsch B. Endocrinology of hirsutism. International journal of trichology. 2010;2(1):30.