Original Research Article

Comparitive study on role of vitamin d supplementation among PCOS women with vitamin D deficiency

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Abstract

Aim & objectives: This study was designed to compare the efficacy of vitamin D supplementation over Metformin in the management of PCOS with Vitamin D deficiency.**Methodology:** The present study is a hospital based prospective study conducted in the department of Obstetrics & Gynaecology Narayana medical college & hospital. 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 D_3 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. Complete physical examination, laboratory tests like serum Total testosterone, serum Fasting insulin for hyperandrogenism & IR respectively & Transabdominal ultrasound scan for ovarian volume was done before starting treatment. **Results:** 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 value 0.0001), where as in group B with Metformin supplementation only 56% showed regularization of cycles. On comparing 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. Regarding BMI, waist hip ratio, acne, hirsutism & serum fasting insulin there was reduction in mean values after treatment for 6months in both groups, but reduction is more in group A(Vitamin D, Calcium & Metformin) than in group B women (Metformin). But these differences was not statistically significant. The abnormal oocyte development in ovary in PCOS is related to a state of meiotic arrest, caused by variations in Calcium &Vitamin D levels leading to menstrual abnormalities & dysregulated body mechanisms. **Conclusion:** The Vitamin D & Calcium are safe, inexpensive & easily available drugs their addition to Metformin can contribute for normalization of the dysregulated metabolism in various tissues including ovaries, pancreas, muscle and enhance the action of Metformin in improving the clinical biochemical features of PCOS. More widespread randomized and controlled empirical attempts seem necessary to determine the potential useful affects of the Calcium, Vitamin D supplementation on different features of the PCOS.

Keywords: Vitamin D, PCOS, Metformin, Hirusitism, Calcium, Serum insulin

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder of women of reproductive age affecting about 6-15% 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

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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, type2 DM, metabolic syndrome, dyslipidaemia, infertility, endometrial hyperplasia, endometrial & ovarian cancers. Early detection and treatment of the disease is absolutely important[1-3].

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.

The optimal management of PCOS is uncertain & treatment focuses on ameoliration of clinical features[4-8].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 possibly decreasing the androgen levels. 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. 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.

Objectives

- Relation between the Vitamin D status and weight (BMI) in PCOS women.
- Association between clinical features, endocrine parameters, metabolicdisturbances and levels of Vitamin D.
- To compare the efficacy of Vitamin D supplementation & Calcium overMetformin in PCOS women in terms of:
- Weight reduction & change in BMI.
- Regularization of menstrual cycles.
- Change in ovarian volume & morphology.
- Decrease in insulin resistance.
- Improvement in cutaneous manifestations of PCOS by subjective &clinical grading.

Data sources

This is a prospective study done in the Department of Obstetrics and Gynaecology, Narayana medical college & Hospital, Nellore for a period of 2 years (Oct 2014-Oct 2016).

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, Narayana Medical College & Hospital, Nellore with complaints of menstrual irregularities/ hirsutism/acne arerecruited.

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.

Patient analysis

Institutional ethical committee of Narayana medical college & hospital, Nellore, Andhra Pradesh has approved the study with the following considerations:

1. No bias with respect to age &pre treatment BMI.
2. A written consent to be taken from all subjects after explaining them, regarding the study and then they were included in the study.
3. Confidentiality should be maintained.

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 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.

Variables considered were

Weight, BMI, W-H ratio, Acne, Hirsutism, sr.Total testosterone, sr. Fasting insulin, Vitamin D, Ovarian volume

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 Trans 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).

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.

Statistical analysis

The data has been entered into MS-Excel and statistical analysis has been done by using IBM SPSS Version 20.0. 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

Table 1:Age distribution – Group A v/s B

Age (Yrs)	Group – A (n = 50)	(%)	Group – B (n = 50)	(%)	P- Value
16-20	8	16	13	26	
21-25	22	44	18	36	
26-30	20	40	12	24	
31-35	0	0	03	6	
Mean	**24.24 ± 3.34**		**23.4 ± 4.12**		0.266

The above table indicates, majority of women in group A (44%) and in group B (36%) are in age group 21-25 yrs. The mean age of group A & B was 24.24 ± 3.34 & 23.4 ± 4.12 respectively, which is similar.

Table 2:Vitamin D Group – A V/s Group – B

ng/ml	Group – A (n = 50)	(%)	Group – B (n = 50)	(%)	P- Value
<10	19	38	10	20	
11-15	17	34	25	50	
16-20	13	26	15	30	
> 21	1	2	0	0	
Mean	**12.32 ± 4.37**		**13.14 ± 4.34**		0.349

As per the above table 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.

Table 3: BMI before Treatment – Group A v/s B

BMI (Kg/m^2)	No. of Cases	Group – A (n = 50)	(%)	Group – B (n = 50)	(%)	P- Value
< 20		3	6	3	6	
21-25		16	32	21	42	
26-30		25	50	21	42	
31-35		11	22	5	10	
Mean		**26.63 ± 3.48**		**25.95 ± 3.69**		0.348

As per above figure, 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.

Table: 4 BMI After Treatment – Group A v/s B

BMI (Kg/m^2)	No. of Cases	Group – A (n = 50)	(%)	Group – B (n = 50)	(%)	P- Value
< 20		3	6	4	8	
21-25		28	56	26	52	
26-30		18	36	17	34	
31-35		1	2	3	6	
Mean		**24.51 ± 2.90**		**24.82 ± 3.47**		0.634

As per the above figure, at the end of treatment,56% in group A & 52 % in group B are in the BMI ranging between 21-25 kg/m^2.The mean BMI in group A & B was 24.51 ± 2.90 & 24.82 ± 3.47 respectively & the reduction in BMI was more in group A after treatment.

Table 5: Waist – Hip Ratio Before Treatment – Group A v/s B

Waist Hip	Group – A (n = 50)	(%)	Group – B (n = 50)	(%)	P- Value
< 0.8	6	12	11	22	
0.81 to0.86	25	50	20	40	
> 0.87	19	38	19	38	
Mean	**0.85 ± 0.38**		**0.84 ± 0.44**		0.233

As per the above figure, 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.

Table 6: Waist – Hip Ratio After Treatment – Group A v/s B

Waist Hip Ratio	Group – A		Group – B		P- Value
	(n = 50)	(%)	(n = 50)	(%)	
< 0.8	10	20	18	36	
0.81 to0.86	34	68	24	48	
> 0.87	6	12	8	16	
Mean	**0.82 ± 0.36**		**0.82 ± 0.43**		0.941

As per the above figure, 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 7: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	
Irregular	17	34	20	40	
Oligo	19	38	25	50	
Regular	2	4	0	0	0.114

As per the above figure, 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.

Table 8: Menstrual abnormalities After Treatment – Group A v/s B

Menstrual abnormalities	Group – A		Group – B		P-Value
	(n = 50)	(%)	(n = 50)	(%)	
Irregular	4	8	13	26	
Oligo	5	10	8	16	
Regular	41	82	28	46	
No change	0	0	1	1	0.031

As per the above table, 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 9: 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		11	22	11	22	
Absent	30	60	28	46	0.685	39	78	39	78	1.000

The above figure shows, 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 10: HirsutismBefore Treatment – Group A v/s B

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

As per the above table, 50% in group A & 48% in group B was noted with hirsutism. In group A 36% & in group B 21%were mild hirsuits with score13-15. 24% in group A & 21% in group 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 11:HirsutismAfter Treatment – Group A v/s B

Hirsutism Score	Group – A		Group – B		P- Value
	(n = 50)	(%)	(n = 50)	(%)	
7-12	22	88	19	73	
13-15	3	12	3	12	
> 15	0	0	4	15	
Mean	**9.68 ± 2.2**		**11.15 ± 3.12**		0.58

As per the above table, at the end of the treatment women with severehirsutism was zero in group A & 15% in group B .Women with moderate

hirsutism in group A & B are 12%. The mean score was in group A 9.68 ± 2.2 & in group B was 11.15 ± 3.12. There was better reduction in group A, but was not statistically significant.

Table: 12 Ovarian Volume Before Treatment – Group A v/s B

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

The above figure shows, majority (62%) in group A & 60% in group B werewith 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 13: Ovarian Volume After Treatment – Group A v/s B

Volume inCm^3	Group – A		Group – B		P- Value
	(n = 50)	(%)	(n = 50)	(%)	
<7	37	74	22	44	
7-10	13	26	28	56	
> 10	0	0	0	0	
Mean	**6.64 ± 1.064**		**7.48 ± 1.07**		<0.0001

The above figure shows, at the end of the treatment the majority of women in group A (74%) have ovarian volume < 7 cm^3, where as majority in group B (56%)had volume between 7-10cm^3.The mean volume in group A was 6.64 ± 1.064 & in group B was 7.48 ± 1.07and the difference in ovarian volume was statistically significant with p value <0.0001 showing addition of Vitamin D & Calcium to Metformin is beneficiary.

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.

The management of PCOS depends on the symptoms either ovulatory dysfunction – related menstrual disorders or androgen related symptoms

Correction of BMI and change in life style are the prior steps in the treatment of PCOS.Weight loss improves the endocrine profile & increases the likelihood of ovulation. Initial weight loss by even 5% can modestly normalize the cycles and ovulation[9].

An increased physical activity is recommended although this often presents limitations. Bariatric surgery has been advocated as a weight loss in the morbidly obese who failed to loose 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[10]

Oral contraceptive pills reduce hyperandrogenism by promoting direct negative feedback on LH secretions, resulting in decreased ovarian androgen synthesis. Also increases the production of SHBG by liver. All these normalize the menstrual cycles & ovarian parameters. Newer progestins which are less androgenic like Drosperinone, Cyproterone acetate will have more advantages.

Surgical ovarian drilling either by diathermy either by laser or electro cautery reduces the local androgens & stromal thickness. It seems to reduce the androgens & regularize the menstrual cycles & ovulation.

Low levels of Vitamin D that causing dysregulation of Calcium metabolism may be the primary factors in the intiation & development of PCOS, and the dietary repletion of this important Vitamin could help to restore normal menstrual cycles in women with PCOS[2]. Calcium plays a major role in oocyte maturation in mammals. Calcium regulation intracellular & extracellular which is essential for transport of glucose in targettissues is indirectly regulated by Vitamin D.

Vitamin D deficiency causes IR and diabetes which induce hyperandrogenism followed by menstrual irregularity &ovulatory dysfunction affecting the fertility of women[9-12].

The potential mechanisms by which Vitamin D can affect glucose metabolism could be the result of either direct or indirect actions of serum 25OH-D

- Direct stimulation of insulin release through the expression of VDR as well as the
- enzyme 1α-OHase in the pancreatic β cells
- Through binding of the 1,25(OH)2D-VDR complex to the vitamin D response elements of the INSR at the tissue level and thereby enhancing insulin responsiveness for glucose transport
- Suppression of proinflammatory cytokines that are belived to mediate IR.

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

There was inconsistency in the results of studies analyzing the relationship between vitamin D status and metabolic disturbances in PCOS women. These conflicting findings are due to small sample sizes, lack of adjustments for confounders, using different diagnostic criteria, different assays for vitamin D measurements & differences in duration & dosage of supplementation in different trials. However a large randomized controlled studies are required for achieving the more conclusive results regarding the effectiveness of Vitamin D & Calcium supplementation in addition to Metformin.

In this present study, we evaluated the effect of Metformin and combination of vitamin D, Calcium to Metformin on menstrual regularity, symptoms of hyperandrogenism, IR & ovarian volume in 100 PCOS women with vitamin D deficiency after 6 months of supplementation. The results showed combination of Vitamin D, Calcium to Metformin seems to have significant effects. Though Metformin therapy also had a significant effect, the combination seems to be more potent specially in menstrual regularization, decreasing ovarian volume & thus improving the fertility rates. Our study is limited by small sample size, shorter duration and limit resources. More widespread randomized and controlled empirical attempts seem necessary to determine the potential useful affects of the Calcium, Vitamin D supplementation on different features of the PCOS.

Age distribution

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. Our study correlated (mean age - 24.24) well with study done by Rashidi et al[2]

Levels of vitamin D in study

The above studies shows the prevalence of Vitamin D deficiency is high in PCOS women. **GunjanGarg et al**(2015) [1] showed in their study 93.8% prevalence of Vitamin D. **E.Wehr et al** [al] 72.8% respectively PCOS women are Vitamin D deficient in their studies. Whereas **Thys Jacob et al** (1998) [5] showed 85% Vitamin D deficient women in their study.

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.

Vitamin D, being a fat soluble vitamin sequestrates in high proportion in adipose tissues lowering the bioavailability in PCOS women especially in obese PCOS women, which leads to high prevalence of Vitamin D deficiency in PCOS women.

BMI (kg/m^2)

There was a reduction by 4.6 % in mean BMI by 3 months of treatment itself. In present study the reduction of mean BMI was 4% [11]

While comparing the treatment efficacies in both the groups (A & B) the mean BMI reduction was 8% in group A & 4% in group B showing no significant differences between the two groups with p value 0.233. But the reduction is more in group A showing addition of Vitamin D & Calcium to Metformin will show better reduction in weight & BMI.

Waist hip ratio

There are no documented studies till date available showing the positiveeffect 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.Present study showed reduction in mean waist hip ratio by 2.5% after 6 months of treatment which is in par with **previous** study (2%) [12]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.

Hirsutism

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

The present study showed a reduction by 28% in hirsutism positive women after treatment for 6months which is comparable with the study of **previous one[9].**

Menstrual cycles

Calcium homeostasis is regulated by Vitamin D, by altering Calcium signaling pathway increases in Calcium influx .This helps in activation & maturation of oocytes inturn results in progression of follicular development & ovulation leading to normalization of menstrual cycles

Studies done by **E.Wehr et al** (2011) showed an regularisation of menstrual cycles in 66% & 39.5% of patients respectively after 6 months of treatment with Vitamin D , Calcium and Metformin.

Previuos studies nshowed a improvement in menstrual cycles after treatment with Metformin for 6 months with 50% & 57.5% respectively.

The present study, showed an improvement in regularization of cycles by 56% after 6 months of treatment which is in par with other studies & is in close relation with **previous study.** 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.

Acne

As per the above mentioned studies, showed reduction of percentage of women with acne by 50% after treatment, for 4 & 6 months respectively.

The present study showed reduction by 45% withstatistically insignificant p value for acne positive cases after treatment, which is in par with **other study**

The present study showed an 50% improvement in acne positive cases after 6 months of treatment which is comparable with **other** study.

On comparing the efficacy of two groups, group A showed 45% & group B showed 50% reduction in acne positive cases .This shows no significant differences between the two groups regarding acne reduction[9-11]

Ovarian volume

Vitamin D & Calcium supplementation regulates the impaired Calcium regulatory system which promotes oocyte maturation , resumption& progression of follicular development, resulting in dominant follicle formation & decreases ovarian volume**.**

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

Rashidi et al in 2008 showed an improvement by 36.8% in the growth of follicles (10-14mm) in group received Metformin, Vitamin D & Calcium when compared to other groups received either Metformin alone or Calcium & Vitamin D alone.

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.

Present study showed a reduction in ovarian volume by 13% which is in par with other studies.

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 <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.

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**.**

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