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A Comparative Effect of NAC, Metformin And Vitamin D3 with Calcium on Reproductive Hormones in PCOS

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Abstract
Objective *– This study was aimed to evaluate the effect of N-Acetyl cystein, metformin and vitamin D3 with calcium on reproductive hormones in PCOS.*
Material And Methods*: The present study was conducted in the department of obstetrics and gynecology ,Motilal Nehru medical college, Allahabad ,in 66 women of reproductive age group. The diagnosis of PCOS was made if the patients had two out of three Rotterdam criteria.*
Result: *After 3 months of treatment, s. total testosterone and s.LH/FSH ratio decreased significantly in all the three groups (p=0.0001). The difference in mean value of s. testosterone between three groups before treatment was statistically insignificant (p=0.2254). However post treatment, significant difference between the groups was observed (p=0.0). The difference in mean value of s.LH/FSH between three groups before treatment was statistically insignificant (p=0.194). However post treatment, significant difference between three groups was observed (p=0.0318).*
The decrease in free androgen index between the groups receiving N-acetyl cysteine and Metformin (p>0.05) was insignificant and groups receiving Metformin and Vitamin D3 with Calcium (p>0.05) was also insignificant. However, the decrease in free androgen index in group receiving N-acetyl cystien was more than the group receiving Vitamin D3 with Calcium.
However post treatment, significant difference between three groups was observed(p<0.0308). The difference of mean between Group 1 and Group 2 was statistically insignificant (p>0.05) and between Group 1 and Group 3 was also statistically insignificant (p>0.05). However, the difference between Group 2 and Group 3 was statistically significant (p<0.05).
The increase in s. SHBG in group receiving Metformin was more than group receiving N-acetyl cysteine an Vitamin D3 with Calcium (52.97± 4.45 from 26.76 ± 3.11 vs 51.18 ± 3.41 from 24.05 ± 5.90 and 46.45 ± 6.75 from 26.50 ± 5.23 respectively).
Conclusion: *In all 3 groups, total testosterone and free androgen index decreased and sex hormone–binding globulin increased. Greater improvement in all these measures was seen in N-acetyl cysteine than others two groups. However, sex hormone–binding globulin increased more in the Metformin group in comparison to both N-acetyl cysteine and Vitamin D3 with Calcium groups.*
Keywords: *PCOS, n-acetyl cystein, metformin,vitamin D3, S.Testosterone, S.FSH/LH, S.SHBG*

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I. Introduction

In India more than 25% reproductive women are suffering from PCOS. It is characterized by the combination of hyperandrogenism, chronic anovulation and polycystic ovaries with the clinical manifestation of oligomenorrhea /amenorrhea, acne, hirsutism, and infertility . The characteristic polycystic ovary develops when a chronic anovulatory state persist for a long time. A cross section of anovulatory women at any one point in time will demonstrate that approximately 75 % have multicystic ovaries. Hormonal profile of PCOS compared to normal cycling women exhibit increased serum luteinizing hormone (LH) concentration and increased LH:FSH ratio.The increased LH level results from abnormal LH secretory dynamics, characterized by increase in LH frequency, and to lesser extent, also in pulse amplitude. The decrease in FSH level result from increase in GnRH pulse frequency, the negative feedback effects of chronically elevated estrone concentrations (derived from peripheral aromatization of increased androstenedione) and normal or modestly increased level of inhibin B (derived from small follicles) (Leon speroff pg 2015 [1]

According to the Rotterdam criteria (2003)***(Rotterdam et al)***[2] PCOS is diagnosed, if there is presence of two out of the following three criteria.

1. Oligomenorrhoea and/or anovulation.
2. Hyperandrogenism (clinical and/or biochemical).
3. Ultrasound appearance of Polycystic ovaries, with the exclusion of other etiologies.

The sonographic criteria for PCOS require the presence of 12 or more follicles in either ovary measuring 2-9 mm in diameter and/or increased ovarian volume (>10ml) a single ovary meeting these criteria sufficient to affix the PCOS diagnosis Hyperandrogenism and anovulation that accompany PCOS maybe , caused by abnormalities in four endocrinologically active compartments***(Rosenfield RL et al)***[3]**.** (1)ovaries, (2) adrenal glands, (3)periphery (fat), (4)hypothalamus-pituitary compartment in patients with PCOS. The ovarian compartment is the most consistent contributor of androgen. Dysregulation of CYP 17, the androgen forming enzyme in both the ovaries and adrenal gland is one of the central pathogenic mechanism underlying hyperandrogenism in PCOS. The ovarian stroma, theca and granulosa cells contribute to ovarian hyperandrogenism and are stimulated by LH. The ovaries are more sensitive to gonadotrophin stimulation, possibly as a result of CYP 17 dysregulation.

NAC (N-acetyl-cysteine) is a stable derivative of the amino acid cysteine, which has antioxidant properties and is required for the body's production of glutathione. Glutathione along with NAC are powerful antioxidant. Through acceleration of Glutathione synthetase hormone (GSH) synthesis*(MS Soltan-Sharifi et al)*[4]there occurs inhibition of oxidative stress and consequently the prevention of hyperinsulinemia induced insulin resistance and preservation of insulin receptors against oxidant agents*(Fulghesu AM et al)*[5] Metformin,(molecular formula $C_4H_{11}N_5$) is an oral antidiabetic drug in the biguanide class. It is an insulin sensitizer and works by suppressing glucose production by the liver. It is the first-line drug of choice for the treatment of type 2 diabetes, in particular, in overweight and obese people and those with normal kidney function.Treatment with metformin might indeed decrease the risk of developing diabetes and heart disease in women with PCOS. The most logical candidates for treatmen with metformin are women with impaired glucose tolerance or diabetes, those with obvious evidence of severe insulin resistance and women having other features of metabolic syndrome such as central obesity, hypertension and dyslipidemia.

The most beneficial serum concentrations of 25(OH) vitamin D are observed at levels >30ng/ml. Most experts agree that vitamin D insufficiency is present with 25(OH) vitamin D levels of 21-29ng/ml and deficiency levels <20ng/ml**(Chue et al 2004)[6].**Vitamin D deficiency is now a globally recognized pandemic. The main cause of vitamin D deficiency is lack of exposure to sunlight, use of sunscreen, melanin pigmentation, winter, latitude, malabsorption and use of medications like glucocorticoids, rifampicin, anti seizure medication, and retroviral therapy.

Aim & Objectives

The present study was undertaken in women having characteristics of PCOS, with the following aims & objectives-

- To determine the effect of N_acetyl cysteine(NAC), Metformin and Vitamin-D3 with Calcium onLH/FSH ratio, total testosterone, free androgen index and sex hormone–binding globulin(SHBG).

II. Material And Methods

The present study was done in the Swaroop Rani Nehru hospital, Department of Obstetrics and Gynaecology, MLN Medical College,Allahabad The study was carried out in 66 women of reproductive age group, with characteristics of PCOS like oligomenorrhoea, amenorrhea, clinical and/or biochemical signs of hyperandrogenism and/or obesity, polycystic ovarian morphology on Ultrasonography i.e., presence of 12 or more follicles in either ovary measuring 2 to 9 mm in diameter and increased volume, >10 ml. of a single ovary.Cases were defined as having PCOS according to Rotterdam criteria. The diagnosis of PCOS was made if the patients had two out of three criteria. Women with systemic and endocrine disorders, late onset Congenital Adrenal Hyperplasia, Cushing's Syndrome, Thyroid dysfunction, Hyper prolactinemia, diabetes mellitus, Coronary heart disease, on medication known to alter insulin hemodynamic, Ovulation index, OCPs and anti-obesity drugs within three months were excluded from study.

History of obesity, palpitations, hypertension, cardiovascular risk factors, and impaired glucose tolerance was taken. The patients were also asked about history of diabetes-mellitus, thyroid disorder, congenital adrenal hyperplasia and adrenal tumours. Detailed family history regarding PCOS in the first and second degree relatives and personal history were also taken..In married women per speculum examination was done for inspecting vagina and cervix and bimanual per vaginum examination for size and direction of uterus. Hormonal assessments was done at baseline and repeated after 12 weeks of treatment.

Serum FSH, serum LH and serum Testosterone was measured by chemiluminescent enzyme immunoassay.SHBG by immune enzymometric assay

Normal values of these hormones on D2 were taken as --

- **Serum FSH** 3-12mIU/ml.
- **Serum LH Level** 3-15 mIU/ml
- The minimum detectable concentration of human Luteinizing Hormone by PATHOZYME LH was estimated to be 1mlU/ml.
- (LH/FSH) >2 suggest PCOS: Measured by bioassay.
- **Serum Total Testosterone-** 0.2 to 0.8 ng /ml
- **SHBG**- 40-120 nmol/l in adult females.

Subjects were randomly assigned to one of three treatment groups. Group 1 was given NAC,600 mg three times a day. Group 2 Metformin hydrochloride, 500 mg two times a day for 1 week, then three times a day for rest of the study and. Group 3 vit-D3 60,000 IU weekly and Calcium 1500mg daily.

III. Result

66 cases were divided into three groups. Each group had 22 patients. Group 1received NAC,600 mg three times a day,Group 2 Metformin hydrochloride 500 mg two times a day for one week, then three times a day for rest of study and Group 3 Vit-D3 60,000 IU weekly and Calcium 1500mg daily. Reproductive hormones parameters were measured before treatment and after 12 weeks of treatment.

IV.Observations

The present study was carried out on women between 18-35 years of age attending the outpatient department. All women fulfilling Rotterdam criteria for PCOS after taking detailed history and thorough clinical examination were subjected to investigations and transvaginal ultrasonography. serum total testosterone, serum sex hormone binding globulin and free androgen index were measured at first visit and after 12 weeks of study.Total 66 cases were taken with 22 cases in each group .The cases in Group 1received NAC,600 mg three times a day,Group 2 received Metformin hydrochloride 500 mg two times a day for one week, then three times a day for rest of study and Group 3 received Vit-D3 60,000 IU weekly and Calcium 1500mg daily. The

reproductive hormone levels were again measured after 12 weeks of study. The effect of treatment was observed on reproductive hormone levels was observed.

Table 1: Serum Total Testosterone (Pre Tt and post Tt)

Serum Total Testosterone (nmol / l)	Group 1				Group 2				Group 3			
	Pre Tt		Post Tt		Pre Tt		Post Tt		Pre Tt		Post Tt	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
0.7-2.8nmol/l	18	81.81	22	100	19	86.36	21	95.45	18	81.81	20	90.90
>2.8nmol/l	4	18.18	0	0	3	13.63	1	4.54	4	18.18	2	9.09
Total	22	100	22	100	22	100	22	100	22	100	22	100
Mean ± SD	2.05 ±0.67		1.41 ± 0.66		2.03 ± 0.67		1.60 ±0 .58		2.08 ± 0.68		1.70 ± 0.65	

Before treatment in Group 1, 18 cases (81.81 %) had s .total testosterone was 0.7-2.8 nmol /l and 4 cases (18.18%) had s. total testosterone >2.8 nmol/l with the mean of 2.05 ± 0.67 nmol/l. In Group 2, 19 cases (86.36%) had s .total testosterone was 0.7-2.8 nmol/l and 3 cases (13.63 %) had s. total testosterone>2.8 nmol/l with the mean of 2.03 ± 0.67nmol/l. In Group 3, 18 cases (81.81 %) had s .total testosterone was 0.7-2.8 nmol/l and 4 cases (18.18 %) had s. total testosterone >2.8 nmol/l with the mean of 2.03 ±0.67nmol/l. After 12 weeks of treatment, in Group 1, all the 22 cases (100%) had s. total testosterone 0.7-2.8 nmol/l with the mean of 1.41 ± 0.34 nmol/l. In Group 2, 21 cases (95.45 %) had s. total testosterone 0.7-2.8 nmol/land 1 case had total testosterone>2.8 nmol/ml with the mean of 1.60± 0.58 nmol/l. In Group 3, 20 cases (90.90 %) had s. total testosterone 0.7-2.8 nmol/l and2 cases (9.09 %) had s. total testosterone > 2.8nmol/l with the mean of 1.70 ± 0.65 nmol /l.

Table2: Effect on Serum Total Testosterone

Groups	Pre Tt	Post Tt	Paired test p value
Group 1	2.05±0.67	1.41±0.66	0.0001
Group 2	2.03±0.67	1.60±0.58	0.0001
Group 3	2.08±0.68	1.70±0.65	0.0001
ANOVA test p value	0.9702	0.2141	

After 3 months of treatment, s. total testosterone decreased significantly in all the three groups (p=0.0001). The difference in mean value between three groups before treatment was statistically insignificant (p=0.2254). However post treatment, significant difference between three groups was observed (p=0.0

Table 3 LH/FSH (Pre Tt and post Tt)

LH/FSH Ratio	Group 1				Group 2				Group 3			
	Pre Tt		Post Tt		Pre Tt		Post Tt		Pre Tt		Post Tt	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
<2	13	59.09	17	77.27	10	45.45	14	63.63	8	36.36	10	45.45
>2	9	40.90	5	22.72	12	54.54	8	36.36	14	63.63	12	54.54
Total	22	100	22	100	22	100	22	100	22	100	22	100
Mean ± SD	1.5 1.58±0.59		1.33±0.53		1.81 ± 0.56		1.61 ±0 .53		1.87 ± 0.55		1.76 ± 0.56	

Before treatment in Group 1, 13 cases (59.09 %) had s .LH/FSH ratio <2and9cases (40.90%) had s. LH/FSH ratio >2with the mean of 1.58 ± 0.59. In Group 2, 10 cases (45.45%) had s .LH/FSH ratio<2 and 12cases (54.54%) had s. LH/FSH ratio >2 with the mean of 1.81 ± 0.56. In Group 3, 8 cases (36.36%) had s .LH/SH ratio <2 and 14cases (63.63%) had s. LH/FSH >2 with the mean of 1.87 ±0.55. After 12 weeks of treatment, in Group 1, 17cases (77.27 %) had s. LH/FSH ratio0<2 and 5 cases (22.72 %) had s. LH/FSH ratio >2with the mean of 1.33 ± 0.53 . In Group 2, 14 cases (63.63%) had s. LH/FSH ratio<2 and 8 cases (36.36%) had s. LH/FSH ratio >2 with the mean of 1.61± 0.53. In Group 3, 10 cases (45.45%) had s. LH/FSH ratio <2and 12 cases (54.54 %) had s. LH/FSH ratio> 2 with the mean of 1.76 ± 0.56.

Table 4 Effect on LH/FSH Ratio

Groups	Pre Tt	Post Tt	Paired test p value

Group 1	1.58±0.59	1.33±0.53	0.0001
Group 2	1.81±0.56	1.61±0.53	0.0001
Group 3	1.87±0.55	1.76±0.56	0.0001
ANOVA test p value	0.194	0.0318	

After 3 months of treatment, s.LH/FSH ratio decreased significantly in all the three groups (p=0.0001). The difference in mean value between three groups before treatment was statistically insignificant (p=0.194). However post treatment, significant difference between three groups was observed (p=0.0318).

Table 5: Free Androgen Index (Pre Tt and post Tt)

Free Androgen Index)	**Group 1**				**Group 2**				**Group 3**			
	Pre Tt		**Post Tt**		**Pre Tt**		**Post Tt**		**Pre Tt**		**Post Tt**	
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
<7 %	5	22.72	22	100	5	22.72	22	100	3	13.63	22	100
7-10 %	17	77.27	0	0	17	77.27	0	0	19	86.36	0	0
Total	22	100	22	100	22	100	22	100	22	100	22	100
Mean ± SD	7.93 ± 0.82		3.02 ±0.68		7.7 ± 0.86		2.58 ± 0.44		7.81 ± 0.64		3.03 ± 0.61	

Before treatment in Group 1, 5 cases (22.72%) had free androgen index < 7% and 17 cases (77.27%) had free androgen index 7-10 % with the mean of 7.93 ± 0.82 %. In Group 2, 5 cases (22.72%) had free androgen index < 7% and 17 cases (77.27%) had free androgen index 7-10 % with the mean of 7.7 ± 0.86 %. In Group 3, 3 cases (13.63%) had free androgen index <7% and 19 cases (86.36%) had free androgen index 7-10 % with the mean of 7.81 ± 0.64 %. After treatment, in Group 1, 22 cases (100%) had free androgen index <7% with mean of 3.02 ± 0.68 %. In Group 2, 22 cases (100%) had free androgen index <7% with mean of 2.58 ± 0.44. In Group 3, 22 cases (100%) had free androgen index <7% with mean of 3.03 ± 0.61.

Table 6: Effect on Free Androgen Index

Groups	**Pre Tt**	**Post Tt**	**Paired test p value**
Group 1	7.93±0.82	3.02±0.68	0.0001
Group 2	7.7±0.86	2.58±0.44	0.0001
Group 3	7.81±0.64	3.03±0.61	0.0001
ANOVA test p value	0.6501	0.0308	

The post treatment the free androgen index decreased significantly in all the three groups (p=0.0001). The difference in mean value between three groups before treatment was statistically insignificant (p=0.6501). However post treatment, significant difference between three groups was observed(p<0.0308). The difference of mean between Group 1 and Group 2 was statistically insignificant (p>0.05). The difference of mean between Group 1 and Group 3 was statistically insignificant (p>0.05). However, the difference between Group 2 and Group 3 was statistically significant (p<0.05).

Table 7 S.SHBG (Pre Tt and post Tt)

S.SHBG (nmol/l)	**Group 1**				**Group 2**				**Group 3**			
	Pre Tt		**Post Tt**		**Pre Tt**		**Post Tt**		**Pre Tt**		**Post Tt**	
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	
<20	2	9.09	0	0	0	0	0	0	2	9.09	0	0
20-40	20	90.90	0	0	22	100	1	4.54	19	86.36	4	18.18
>40	0	0	22	100	0	0	21	`95.45	1	4.54	18	81.81
Total	22	100	22	100	22	100	22	100	22	100	22	100
Mean±SD	24.05± 5.90		51.18± 3.41		26.76± 3.11		52.97± 4.45		26.50± 5.23		46.45± 6.75	

Before treatment, In Group 12 cases (9.09 %) had s. SHBG <20nmol/ml,20 cases (90.90) had s.SHBG 20-40 nmol/l with the mean of 24.05 ± 5.90. In Group 2 , all the 22 cases (100%) had s. SHBG 20-40 nmol/l with the mean of 26.76 ± 3.11. In Group 3, 2 cases (9.09 %) had s. SHBG<20nmol/ml,19 cases(86.36) had

s.SHBG 20- 40 nmol/l , 1 case had s.SHBG>40 nmol/ml with the mean s. SHBG of 26.50 ± 5.23nmol/l. After treatment, In Group 1 all 22 cases(100%) had, s.SHBG>40 nmol/ml with mean of 51.18±3.41. In Group 2, 1 case had s.SHBG 20-40 nmol/ml, 21 cases (95.45%) had s.SHBG >40 nmol/ml with mean of 52.97± 4.45. In Group 3, 4 cases (18.18%) had s. SHBG 20-40 nmol/ml, 18 cses (81.81%) had s.SHBG >40 nmol/ml with mean of46.45±6.75.

Table 8: Effect on S.SHBG

Groups	Pre Tt	Post Tt	Paired test p value
Group 1	24.05 ± 5.90	51.18 ± 3.41	0.0001
Group 2	26.76 ± 3.11	52.97± 4.45	0.0001
Group 3	26.50 ± 5.23	46.45 ± 6.75	0.0001
A test p value	0.1323	0.0002	

The increase in s. SHBG in three groups was statistically significant (p=0.0001). The difference between the groups was statistically insignificant pre-treatment (p=0.1323). After 12 weeks of therapy, the difference of mean s. SHBG was statistically significant (p=0.0002). The difference between group 1 and group 2 was statisticallyiinsignificant (p < 0.1743) .The difference between group 2 and group 3 was statistically significant (p<0.0001). The difference of mean s. SHBG between group 1 and group 3 was statistically significant.(p<0.0001).

IV. Discussion

In accordance to present study **Gayatriet al (2010)**[7]evaluated the effects ofN-acetyl cysteine on the metabolic features of women with PCOS and compared with Metformin. After 3 months of treatment, Serum LH level was decreased in both the groups, but it was more significant in NAC group. Change in serum FSH level and LH/FSH ratio was not significant in Metformin group, where as it was significant in NAC group. TT was decreased in both the groups, but in NAC group it was more significant. Nevertheless, no significant change was observed in serum FT. There was no significant change in s.SHBG in both the groups. In contrast, **Salehpur et al (2009)**[8]**,** found no significant change in serum LH,FSH, and LH/FSH ratio. But a significant decline in post treatment s.TT as observed.

Table 9: Effect of N-acetyl cysteine on Reproductive hormone parameters

Study	Year	Duration of study	N-acetyl cysteine	Clinical Parameters	Mean±SD	P Value
Gayatri et al	2010	12 weeks	600 mg1TDS.	s. TT (nmol/l)	1.15±0.21 (from1.65±0.24)	<0.001
				s.SHBG (nmol/l)	62.39±20.21(from 61.35±20.48)	>1
				LH/FSH ratio	2.19±0.71(from 2.49±0.76)	<0.02
Salehpour et al	2009	6 weeks	600 mg 1 TDS	s.TT (nmol/l)	0.76±0.42 (from0.91±0.48)	0.011
				LH/FSH ratio	0.95±0.38(from 1.08±0.46)	0.473
Present study	2015-16	12 weeks	600mg 1TDS for12 weeks	s.TT (nmol/l)	1.41±0.66 (from2.05±0.67)	0.0001
				s.SHBG (nmol/l)	60.07±4.63(from 25.46±2.24)	0.0001
				LH/FSH ratio	1.33±0.53(from 1.58±0.59)	0.0001
				FAI %	3.02±0.68 (from7.93±0.82)	0.0001

Table 10 : Effect of Metformin on Reproductive hormone parameters

Study	Year	Duration of study	Metformin Doses	Clinical Parameters	Mean±SD	P Value
Gayatri et al	2010	12 weeks	600 mg1TDS.	s. TT (nmol/l)	1.35±0.29 (from1.55±0.29)	0.05
				s.SHBG (nmol/l)	62.21±20.26(from 61.29±20.32)	>1
				LH/FSH	2.61±0.7(from 2.64±0.72)	>1
Present study	2014-15	12 weeks	500 mg BD For1weeks, then	s.TT (nmol/l)	1.53±0.51 (from1.89±0.66)	0.0001

three times a day for rest of study	s.SHBG (nmol/l)	60.07±4.63(from 25.46±2.24)	0.0001
	LH/FSH ratio	1.61±0.53(from 1.81±056)	0.0001
	FAI%	2.58±0.44 (from7.7±0.86)	0.0001

Similarly,**Pikee Saxena et al (2010)[9]** conducted a study to known the effect of Metformin on clinical, hormonal and metabolic parameters. Metformin therapy showed significant reductions in serum LH and testosterone level . Serum FSH level was unaltered.

In contrast, **Gayatriet al (2010)[7]**found that , after 3 months of treatment,LH/FSH ratio and s. SHBG was not significantly decreased in Metformin group.. TT was decreased in both Metformin and N-acetyl cysteine groups, but in NAC group it was more significant.

The study conducted by Velaquez et al (1994)[10]also showed similar findings. The findings of present study were also supported by Yuan et al (2014)[11] who evaluated the clinical, metabolic and endocrine effects of Metformin in PCOS women scheduled for IVF treatment and to explore the potential benefits to the IVF process. The results of their study found that compared with placebo, greater reductions in total testosterone, free androgen index, fasting glucose, fasting insulin and HOMA-IR, and increases in SHBG, were observed in theMetformin groups. In contrast, Selimoglu et al (2010)[12] investigated the effects of vitamin D supplementation on IR, glucose metabolism, and hyperandrogenic features.Three weeks after therapy, analysis showed improvement in serum 25(OH)D levels, decreased in HOMA-IR (P =0.043). Kozakowski J et al (2014)[13] who conducted a study to determine the associations of vitamin D concentration with metabolic and hormonal indices in women with polycystic ovary syndrome presenting abdominal and gynoidal type of obesity. No correlations with androgens were found but in women with abdominal obesity vitamin D deficiency associated with increase luteinizing hormone/follicle-stimulating hormone ratio (LH/FSH) and low SHBG. ChunlaHe et al (2015)[14] conducted a study to assess the associations of serum vitamin D levels with metabolic and endocrine dysregulations in women with PCOS, and to determine effects of vitamin D supplementation on metabolic and hormonal functions in PCOS patients.

The results of this study suggest that supplementation of vitamin D does not significantly improve metabolic (except triglycerides) and hormonal features in PCOS patients. Similar were the findings of Gayatriet al (2010)[7]who compared the effects of N-acetyl cysteine and Metformin on the metabolic and hormonal features of women with PCOS . After 3 months of treatment,. improvement in hormonal parameters was more significant in N-acetyl cysteine group than Metformin. There was no significant changes observed in s.SHBG in both groups. Pikee Saxena et al (2010)[9] conducted a study to known the effect of Metformin on clinical, hormonal and metabolic parameters. Metformin therapy showed significant improvement in hormonal parameters. Similarly, Velaquez et al (1994)[10]who conducted a study to assess the effect of Metformin on lipoproteins, sex hormones and gonadotropins,in women with PCOS. After Metformin therapy there was improvement in insulin sensitivity and significant improvement in hormonal parameters. The findings of present study were also supported by Yuan et al (2014**)[11]**

who evaluated the clinical, metabolic and endocrine effects of Metformin in PCOS. They found that Metformin groups showed comparable changes in total testosterone and free androgen index, which were significantly greater than placebo. In contrast, ChunlaHe et al (2015)**[14]**who conducted a study to assess the associations of serum vitamin D levels with metabolic and endocrine dysregulations in women with PCOS. They concluded that supplementation of vitamin D does not significantly improve metabolic (except triglycerides) and hormonal features in PCOS patients.

V. Conclusion

In this study we concluded that--

1. All the three groups showed insignificant reduction in total testosterone (p=0.2141) However N-acetyl cysteine showed superiority over Metformin and Vitamin D3 and Calcium (1.41±0.66 from 2.05±0.67 vs 1.60 ± 0.0.58 from 2.03±0.67 and 1.70 ± 0.65 from 2.08±0.68)
2. Post treatment reduction in LH/FSH ratio between the three groups was statistically significant(p=0.0318).
3. Greater reduction in LH/FSH ratio was seen in N-acetyl cysteine (1.33±0.53 from 1.58±0.59) than Metformin and Vitamin D3 with Calcium (1.61±0.53 from 1.81±0.56 and 1.76±0.56 from 1.87±0.55).
4. After 3 months of treatment, a significant reduction in free androgen index between the three groups was observed (p=0.0308)
5. The decrease in free andrgen index between the groups receiving N-acetyl cysteine and

Metformin (p>0.05) was insignificant and groups receiving Metformin and Vitamin D3 with Calcium (p>0.05) was also insignificant. However, the decrease in free androgen index in group receiving N-acetyl cysteine was more than the group receiving Vitamin D3 with Calcium.

6. Increase in s. SHBG after three months of supplementation was statistically significant (p=0.0308) .

7. The increase in s. SHBG in group receiving Metformin was more than group receiving N-acetyl cysteine an Vitamin D3 with Calcium (52.97± 4.45 from 26.76 ± 3.11 vs 51.18 ± 3.41 from 24.05 ± 5.90 and 46.45 ± 6.75 from 26.50 ± 5.23 respectively).

Thus the study revealed that longer treatment with N- acetyl cystein may result in more beneficial outcome in terms of hormonal parameters with lesser side results.

Although vitamin D is the basic pathophysiology of pcod but its supplementation with calcium did not give satisfactory results.

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