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Original Research Article

## A study of N-acetyl cysteine, metformin and vitamin D3 with calcium on clinical and metabolic profile in PCOS

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### ABSTRACT

**Background:** Polycystic ovarian syndrome (PCOS) is characterized by the combination of hyperandrogenism, chronic anovulation and polycystic ovaries. Objective of present study was to compare the effects of N-acetyl cysteine, metformin and vitamin D3 with calcium on clinical and metabolic profile in PCOS.

**Methods:** 66 women were randomly assigned into three equal treatment groups. Group 1 received N-acetyl cysteine, 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 with calcium 1500mg daily. Clinical and metabolic assessment was done at baseline and after three months of treatment.

**Results:** After 12 weeks of treatment improvement of symptoms was seen in all the three groups, however better improvement in oligomenorrhea and hirsutism was seen in metformin group than others two groups. The clinical parameters like weight, BMI, waist hip ratio, biochemical markers of insulin resistance, fasting glucose, fasting insulin, fasting glucose/insulin ratio were significantly decreased in N-acetyl cysteine group than others two groups.

**Conclusions:** N-acetyl cysteine had better improvement in clinical, and metabolic profile than metformin and vitamin D3 with Calcium group in PCOS patients. It can be used as a substitute for insulin reducing medications in treatment of PCOS patients, considering its limited adverse effects.

**Keywords:** Clinical and metabolic profile, Metformin, N-acetyl cysteine, PCOS, vitamin D3

### INTRODUCTION

Polycystic ovarian syndrome (PCOS) is characterized by the combination of hyperandrogenism, chronic anovulation and polycystic ovaries. Etiology of the syndrome has remained unknown although it has been revealed that, synthesis of high levels of androgen and insulin-resistance (IR) lies at the core of its pathophysiology (Schuring AN).<sup>1</sup> According to the Rotterdam criteria (Rotterdam et al) PCOS is diagnosed, if there is presence of two out of the following three features: Oligomenorrhoea and/or anovulation, clinical or biochemical sign of hyperandrogenism or both and

polycystic ovaries as seen on ultrasound scanning with the exclusion of other etiologies.<sup>2</sup>

Current incidence of PCOS is increasing fast due to changes in lifestyle and stress. It is also becoming a common problem in adolescent. Some of the women who develop cardiovascular disease, hypertension, endometrial cancer and type 2 diabetes later in life appear to have suffered from PCOS in early years.

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 is a powerful antioxidant. NAC probably influences insulin receptor activity and results in an increase of glucose consumption, which is an indicator of the insulin sensitivity state (Ammon HP et al).<sup>3</sup> Therefore, it was suggested that the above effects exerted by NAC at the ovarian level may be as beneficial as insulin-enhancing effects in inducing ovulation. N-acetyl cysteine reduces plasma homocysteine level (Ventura P et al).<sup>4</sup>

Metformin is an oral antidiabetic drug in the biguanide class. It is an insulin sensitizer and works by suppressing glucose production by the liver. Antidiabetic therapy has been proposed as a treatment for polycystic ovary syndrome (PCOS), a condition frequently associated with insulin resistance. The main effect of this drug is to decrease hepatic glucose production through a mild inhibition of the mitochondrial respiratory-chain complex I. It also increases insulin sensitivity, enhances peripheral glucose uptake by phosphorylation of GLUT 4 enhancer factor.

Vitamin D is a lipid soluble steroid molecule, mainly produced by the skin and a small amount is absorbed in the gut from dietary sources. Currently Vitamin-D deficiency has also been implicated as the cause of insulin resistance and the development of PCOS. 1-25 hydroxy vitamin D is positively correlated with insulin sensitivity and negatively with beta cell function. Vitamin D3 enhances insulin action by enhancing insulin synthesis & release, increased insulin receptor expression and/ or suppression of proinflammatory cytokines that are believed to mediate insulin resistance (McGreevy et al 2011).<sup>5</sup>

## METHODS

The present study was done in the Department of Obstetrics and Gynecology, MLN Medical College, Allahabad over a period of 12 months. The study was carried out in 66 women of age group 15-40 years attending OPD fulfilling the Rotterdam criteria. Women with systemic and endocrine disorders, late onset Congenital Adrenal Hyperplasia, Cushing's Syndrome, Thyroid dysfunction, Hyperprolactinemia, 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.

After approval from the ethical committee and written informed consent detailed history was taken with special reference to age, parity, habitat, socioeconomic status, education, and personal habits such as nutrition and exercise. Special focus was on menstrual pattern such as oligomenorrhoea (interval between menstrual periods  $\geq 35$  days amenorrhoea (absence of vaginal bleeding for at least six months), clinical hyperandrogenism (a Ferriman-gallwey score  $\geq 6$ ) and/or biochemical hyperandrogenism (total testosterone (TT)  $\geq 58$ ng/dl (2nmol/l)) to test for

PCOS. Detailed family history regarding diabetes mellitus, hypertension and PCOS in the first and second degree relatives and personal history were also taken. A thorough clinical examination including general condition, built, height, weight, body mass index, waist/hip ratio, feature of hirsutism (ferriman Gallwey), thyroid gland examination and breast examination for galactorrhoea was done. A detailed systemic examination of central nervous system, cardiovascular system, respiratory system and abdomen was done. In married women per speculum examination was done for inspecting vagina and cervix and bimanual per vaginum examination was also done. Clinical and metabolic assessments were done at baseline and repeated after 12 weeks of treatment.

Subjects were randomly assigned to one of the three treatment groups. Group 1 received N-acetyl cysteine, 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 with Calcium 1500mg daily.

Blood investigations including Serum TSH, Serum Prolactin, Estimation of serum Insulin level, serum fasting glucose level, serum fasting glucose/serum insulin ratio, serum Vitamin D level were done.

After three months of treatment, each subject underwent the same procedure as described above.

## Statistical analysis

The data obtained was analyzed using ANOVA (analysis of variance test). The level of significance in this study was taken as  $p < 0.05$ .

## RESULTS

Majority of cases (70%) were below 30 years of age.

The difference between all the three groups regarding age, marital status, residential area, socioeconomic status, educational status and occupation was found to be statistically insignificant ( $p > 0.05$ ), oligomenorrhoea, hirsutism and infertility improved in all the three groups but was better in N-acetyl cysteine and metformin group than vitamin D3 with calcium (Table 1).

Weight, BMI, waist circumference and waist hip ratio decreased significantly in all the groups ( $p < 0.0001$ ) (Table 1). There were no significant differences in reduction of weight and BMI among three groups ( $p > 0.05$ ) (Table 3). The decrease in waist circumference ( $p = 0.0418$ ) and waist hip ratio ( $p = 0.0001$ ) was significant among three groups. Greater reduction in waist circumference and waist hip ratio was seen in group receiving N-acetyl cysteine group compared to metformin and vitamin D3 with calcium group (Table 2,3).

**Table 1: Comparison of different parameters in each group.**

Parameters	N-acetyl cysteine		Metformin		Vitamin D3 with calcium	
	Pre-treatment (% of cases)	Post-treatment (% of cases cured)	Pre-treatment (% of cases)	Post-treatment (% of cases cured)	Pre-treatment (% of cases)	Post-treatment (% of cases cured)
Oligomenorrhea	72.72%	50%	68.18%	60%	63.63%	35.71%
Infertility	36.36%	50%	27.27%	50%	31.81%	28.57%
Hirsutism	63.63%	57.14%	72.72%	37.505	54.545	41.66%

**Table 2: Comparison of different parameters in each group.**

Parameters	N-Acetyl cysteine			Metformin			Vitamin D3 with calcium		
	Pre treatment	Post treatment	P value	Pre treatment	Post treatment	P value	Pre treatment	Post treatment	P value
Weight	64.54±4.93	62.00±4.95	0.0001	62.72±4.81	60.04±5.24	0.0001	63.5±4.92	61.31±4.75	0.0001
BMI	24.92±2.22	23.96±2.21	0.0001	24.78±2.45	23.84±2.25	0.0001	24.86±2.13	24.00±2.12	0.0001
WC	92.13±7.79	85.09±5.09	0.0001	91.13±5.67	86.63±4.61	0.0001	91.22±4.72	88.59±4.5	0.0001
WHR	0.89±0.044	0.85±0.025	0.0001	0.87±0.036	0.84±0.029	0.0001	0.89±0.038	0.88±0.044	0.0001
FBS	99.45±13.6	87.77±8.58	0.0001	98.68±10.0	92.86±6.95	0.0028	97.95±14.8	93.31±10.3	0.0002
S.FI	17.88±5.39	10.72±5.27	0.0001	19.60±6.31	13.87±5.72	0.0001	20.94±4.81	16.3 ± 4.77	0.0001
FPG/S.FI	4.63±1.26	6.21±2.13	0.001	4.71±1.03	6.71±1.98	0.001	4.68±1.13	6.01±1.68	0.004

**Table 3: Comparison of different parameters in between the groups (N-acetyl cysteine, metformin and vitamin D3).**

Parameters	ANOVA test P value		Remarks
	Pre treatment	Post treatment	
Weight	0.4407	0.5831	Non-significant
BMI	0.42	0.7733	Non-significant
WC	0.8470	0.0418	Significant
WHR	0.1155	0.0001	Significant
FBS	0.929	0.1135	Non-significant
S.FI	0.1854	0.0040	Significant
S.FPG/S.FI	0.2254	0.0035	Significant

Post treatment reduction in fasting plasma glucose, fasting insulin and fasting glucose/insulin ratio was statistically significant in all the three groups ( $p < 0.05$ ) (Table 2). However, no significant reduction were observed between three groups in fasting plasma glucose ( $p = 0.1135$ ) (Table 3). There was significant difference in Serum fasting insulin ( $p < 0.004$ ) and serum fasting glucose/insulin ratio ( $p = 0.0035$ ) between three groups (Table 3). Greater reduction in fasting plasma glucose and fasting insulin was seen in N-acetyl cysteine ( $p = 0.0001$ ) compared to metformin and vitamin D3 with Calcium. However, improvement in fasting plasma glucose/ fasting insulin ratio was greater in metformin group than others two group.

## DISCUSSION

In this study, the mean age of cases in group 1 was  $26.31 \pm 4.08$  years, in group 2 was  $26.5 \pm 4.64$  years and in group 3 was  $25.36 \pm 4.76$  years. The difference between

the three groups was statistically insignificant. Age had significant negative correlation with androgens and positive correlation with fasting glucose, cholesterol, triglyceride and LDL.

Characteristic symptoms of PCOS i.e. oligomenorrhea, hirsutism and infertility improved in all the three groups. Between all the three groups better improvement of hirsutism was seen in N-acetyl cysteine while oligomenorrhea in metformin group (Table1).

Weight reduction, decrease in BMI, waist circumference and waist hip ratio is the initial recommendation for patients with PCOS because it promotes health, reduces insulin, and androgen levels, and may restore ovulation. Weight loss, as little as 5% to 7% can reduce free testosterone level significantly.

The decrease in mean weight, body mass index, waist circumference and waist hip ratio after 12 weeks of treatment with N-acetyl cysteine, metformin and vitamin D3 with calcium was statistically significant ( $p = 0.0001$ ) (Table2). While comparing the clinical characteristics between the groups after 12 weeks of therapy the difference in weight and BMI was insignificant ( $p = 0.5831$  and  $p = 0.7733$  respectively) (Table 3). However, waist circumference decreased significantly between the three groups ( $p = 0.0418$ ), greater reduction in waist circumference was observed in the N-acetyl cysteine group ( $85.09 \pm 5.09$ ) compared to metformin and vitamin D3 with calcium group ( $86.63 \pm 4.61$  and  $88.59 \pm 4.85$  respectively). Waist hip ratio also decreased significantly in all the three groups ( $p = 0.0001$ ), greater reduction in waist hip ratio was observed in N-acetyl cysteine ( $0.85 \pm 0.025$  from  $0.89 \pm 0.044$ ) compared to

metformin and vitamin D3 with calcium group ( $0.84\pm 0.029$  from  $0.87\pm 0.036$  and  $0.88\pm 0.044$  from  $0.89\pm 0.038$  respectively).

The findings of present study was also supported by Gayatri et al evaluate the effects of N- acetyl cysteine in comparison to Metformin (MET) on the metabolic features of women with PCOS.<sup>6</sup> They concluded from the study that subjects treated with N-acetyl cysteine showed significant differences in body weight and BMI compared to the Metformin.

The study conducted by Firouzabadi et al in which subjects having PCOS were randomly assigned in to two treatment groups.<sup>7</sup> Group 1 received 1 500 mg daily of Metformin, and Group 2 was treated with 1000 mg daily of calcium and vitamin D3, 50 000 weekly plus Metformin. They evaluated the menstruation irregularity, changes in BMI and pregnancy rate. The study revealed a more significant decrease in BMI in Group 2 ( $P = 0.054$ ).

Hyperinsulinemia and insulin resistance have proved to be a key link in the generation of the symptoms of PCOS. So, measures of these carbohydrate metabolic parameters throw light on etiology of PCOS and its management. After 3 months of treatment, regarding Fasting Plasma Glucose, the difference in mean value between three groups pre and post treatment remained statistically insignificant ( $p=0.929$  and  $p=0.1135$  respectively) However greater reduction in serum fasting glucose was observed in group receiving N-acetyl cysteine ( $87.77\pm 8.58$  from  $99.45\pm 13.66$ ) as compared to group receiving metformin or vitamin D3 with calcium ( $93.09\pm 7.31$  from  $99.40\pm 11.15$  vs  $93.09\pm 7.31$  from  $99.40\pm 11.15$  respectively). While regarding fasting insulin, significant difference between three groups was observed ( $p<0.0040$ ). However greater reduction in serum fasting insulin was observed in group receiving N-acetyl cysteine ( $10.72\pm 5.27$  from  $17.88\pm 5.39$ ) as compared to group receiving metformin or vitamin D3 with calcium ( $13.87\pm 5.72$  and  $16.31\pm 4.77$ ) respectively. Concerning fasting plasma glucose/ fasting insulin ratio, post treatment, significant difference between three groups was observed ( $p=0.0035$ ). The Group receiving metformin showed superiority over group receiving N-acetyl cysteine and vitamin D3 with calcium (Table 2,3).

Javanmanesh et al conducted a study and similar result were seen.<sup>8</sup> They concluded that NAC improved fasting blood sugar (FBS) and fasting blood insulin better than Metformin.

In contrast, Thakker D et al conducted a study to determine whether NAC therapy was more effective and safe in women with PCOS compared to placebo/metformin.<sup>9</sup> They found that Metformin improved the BMI, insulin level, and lipid levels better than NAC

Alyssa M et al conducted a study to see the improvement of symptoms in patients with polycystic ovarian syndrome by Vitamin D and Calcium Supplementation.<sup>10</sup> They concluded that vitamin D supplementation improves insulin resistance in obese PCOS patients with vitamin D deficiency, and recommends administration of vitamin D supplementation to prevent onset of diabetes mellitus type II or IR development and progression.

## CONCLUSION

In this study lifestyle modification and insulin sensitizers remained the first line therapy of choice and every subject received a particular group of insulin sensitizer for 3-month period. N-acetyl cysteine, Metformin, and Vitamin D3 with Calcium. As compared to the long course of treatment with other insulin reducing medications, the highly significant changes brought by a 12-week course of treatment with NAC suggested that, longer treatment with N-acetyl cysteine may result in more desirable outcomes, such as more effective control of clinical symptoms of PCOS, hyperandrogenism, and carbohydrate parameters. So far less adverse effects have been reported for NAC as it is an amino acid. It was well tolerated by our sample population as compared to Metformin.

The use of metformin in PCOS has received a lot of attention for obvious reasons. The lack of an emphatic or overwhelming efficacy is largely due to the patients' variability in phenotypes and their metabolic parameters. Obtaining an evidence of insulin resistance is a good starting point prior to recommending its use. Based on the available evidence, however, Metformin does not replace the need for lifestyle modification among obese and overweight PCOS women.

Vitamin D deficiency is the basic pathophysiology for PCOS, hence vitamin D3 with calcium supplementation can be tried in the management of PCOS to improve insulin sensitivity. Vitamin D3 also has better patient compliance and well tolerated than Metformin and N-acetyl cysteine. These beneficial effects of Vitamin D3 support a future therapeutic role in women with PCOS.

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