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Clinical assessment of anti-ovulatory infertile women with PCOS by comparative administration of N-acetyl cysteine with metformin

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Abstract
The National Institute of Health (NIH) criteria include clinical and/or biochemical hyperandrogenism and chronic anovulation for diagnosing PCOS whereas the most recent androgen excess and PCOS (AE–PCOS) Society criteria recommend that PCOS should be defined as clinical or biochemical hyperandrogenism associated with ovulatory dysfunction in the form of oligo-anovulation or polycystic ovaries on ultrasound. Insulin resistance and hyperandrogenism is found to play a key role in the pathogenesis of polycystic ovarian syndrome. Hence based on above findings the present study was planned for Clinical Assessment of anovulatory infertile women with PCOS by Comparative Administration of N-Acetyl Cysteine with Metformin. The present study was planned in Department of Obstetrics & Gynaecology, Government Medical College, Bettiah, West Champaran, Bihar, India. The 50 females diagnosed with the polycystic ovarian syndrome (PCOS) were enrolled in the present study. The 25 females were administered with the Metformin and 25 females were administered with the N- Acetyl cysteine (NAC).
The data generated from the present study concludes that NAC improves the clinical features, biochemical markers of insulin resistance, hormonal levels, anovulation and consequently the long-term health status of women with PCOS through inhibition of oxidative stress and improvement of peripheral insulin more effectively when compared with metformin. Due to the lack of adverse effects, NAC can be regarded as an appropriate substitute for insulin-reducing medications in the treatment of PCOS patients.

Keywords: anti-ovulatory, infertile women, PCOS, n-acetyl cysteine, metformin, etc

Introduction
Polycystic ovary syndrome (PCOS) is one of the most common hormonal disorders among women of reproductive age. PCOS is common diagnosis in women presenting with infertility. The exact prevalence of PCOS is not known as the syndrome is not defined precisely. Prevalence of PCOS is highly variable ranging from 2.2% to 26% globally. In few Asian countries prevalence figures are ranging from 2% to 7.5% in China and 6.3% in Srilanka. There are few studies conducted in India. Studies done in South India and Maharashtra, prevalence of PCOS (by Rotterdam's criteria) were reported as 9.13% and 22.5% (10.7% by Androgen Excess Society criteria) respectively.
PCOS was first reported by Stein and Leventhal in 1935, described as symptoms complex with amenorrhea, hirsutism, and enlarged ovaries with multiple cysts.
Polycystic ovary syndrome causes irregular menstrual cycles, excessive body or facial hair and polycystic ovaries as its main symptoms. Polycystic means "many cysts," and PCOS often causes clusters of small, pearl-sized cysts in the ovaries. The cysts are fluid-filled and contain immature eggs. Women with PCOS produce slightly higher amounts of male hormones known as androgens, which contribute to some of the symptoms of the condition.
Early diagnosis of PCOS is important as it has been linked to an increased risk for developing several medical conditions including insulin resistance, type 2 diabetes, high cholesterol, high blood pressure and heart disease. PCOS is an emerging health problem during adolescence therefore promotion of healthy lifestyles and early interventions are required to prevent future morbidities.
Women with polycystic ovarian syndrome (PCOS) have abnormalities in the metabolism of androgens and estrogen and in the control of androgen production. PCOS can result from abnormal function of the hypothalamic-pituitary-ovarian (HPO) axis. A woman is diagnosed with polycystic ovaries (as opposed to PCOS) if she has 20 or more follicles in at least 1 ovary [1].
The major features of polycystic ovarian syndrome (PCOS) include menstrual dysfunction, anovulation, and signs of hyperandrogenism [2]. Although the exact etiopathophysiology of this condition is unclear, PCOS can result from abnormal function of the hypothalamic-pituitary-ovarian (HPO) axis. A key characteristic of PCOS is inappropriate gonadotropin secretion, which is more likely a result of, rather than a cause of, ovarian dysfunction. In addition, one of the most consistent biochemical features of PCOS is a raised plasma testosterone level.
Stein and Leventhal were the first to recognize an association between the presence of polycystic ovaries and signs of hirsutism and amenorrhea (eg, oligomenorrhea, obesity). After women diagnosed with Stein-Leventhal syndrome underwent successful wedge resection of the ovaries, their menstrual cycles became regular, and they were able to conceive. As a consequence, a primary ovarian defect was thought to be the main culprit, and the disorder

came to be known as polycystic ovarian disease.

Further biochemical, clinical, and endocrinologic studies revealed an array of underlying abnormalities. As a result, the condition is now referred to as PCOS, although it may occur in women without ovarian cysts and although ovarian morphology is no longer an essential requirement for diagnosis.

A woman is diagnosed with polycystic ovaries (as opposed to PCOS) if she has 20 or more follicles in at least 1 ovary—measuring 2-9 mm in diameter—or a total ovarian volume greater than 10 cm3 [1].

Women with polycystic ovarian syndrome (PCOS) have abnormalities in the metabolism of androgens and estrogen and in the control of androgen production. High serum concentrations of androgenic hormones, such as testosterone, androstenedione, and dehydroepiandrosterone sulfate (DHEA-S), may be encountered in these patients. However, individual variation is considerable, and a particular patient might have normal androgen levels.

PCOS is also associated with peripheral insulin resistance and hyperinsulinemia, and obesity amplifies the degree of both abnormalities. Insulin resistance in PCOS can be secondary to a postbinding defect in insulin receptor signaling pathways, and elevated insulin levels may have gonadotropin-augmenting effects on ovarian function. Hyperinsulinemia may also result in suppression of hepatic generation of sex hormone–binding globulin (SHBG), which in turn may increase androgenicity [3].

In addition, insulin resistance in PCOS has been associated with adiponectin, a hormone secreted by adipocytes that regulates lipid metabolism and glucose levels. Lean and obese women with PCOS have lower adiponectin levels than do women without PCOS [4].

A proposed mechanism for anovulation and elevated androgen levels suggests that, under the increased stimulatory effect of luteinizing hormone (LH) secreted by the anterior pituitary, stimulation of the ovarian theca cells is increased. These cells, in turn, increase the production of androgens (eg, testosterone, androstenedione). Because of a decreased level of follicle-stimulating hormone (FSH) relative to LH, the ovarian granulosa cells cannot aromatize the androgens to estrogens, which leads to decreased estrogen levels and consequent anovulation. Growth hormone (GH) and insulin-like growth factor–1 (IGF-1) may also augment the effect on ovarian function [5].

Hyperinsulinemia is also responsible for dyslipidemia and for elevated levels of plasminogen activator inhibitor-1 (PAI-1) in patients with PCOS. Elevated PAI-1 levels are a risk factor for intravascular thrombosis.

Polycystic ovaries are enlarged bilaterally and have a smooth, thickened capsule that is avascular. On cut sections, subcapsular follicles in various stages of atresia are seen in the peripheral part of the ovary. The most striking ovarian feature of PCOS is hyperplasia of the theca stromal cells surrounding arrested follicles. On microscopic examination, luteinized theca cells are seen.

Some evidence suggests that patients have a functional abnormality of cytochrome P450c17, the 17-hydroxylase, which is the rate-limiting enzyme in androgen biosynthesis [4].

PCOS is a genetically heterogeneous syndrome in which the genetic contributions remain incompletely described. PCOS is an inherently difficult condition to study genetically because of its heterogeneity, difficulty with retrospective diagnosis in postmenopausal women, associated subfertility, incompletely understood etiology, and gene effect size. Many published genetics studies in PCOS have been underpowered, and the results of published candidate gene studies have been disappointing.

Studies of family members with PCOS indicate that an autosomal dominant mode of inheritance occurs for many families with this disease. The fathers of women with PCOS can be abnormally hairy; female siblings may have hirsutism and oligomenorrhea; and mothers may have oligomenorrhea [6]. Research has suggested that in a large cohort of women with PCOS, a family history of type 2 diabetes in a first-degree family member is associated with an increased risk of metabolic abnormality, impaired glucose tolerance, and type II diabetes [18]. In addition, a Dutch twin-family study showed a PCOS heritability of 0.71 in monozygotic twin sisters, versus 0.38 in dizygotic twins and other sisters [7].

An important link between PCOS and obesity was corroborated genetically for the first time by data from a case-control study in the United Kingdom that involved 463 patients with PCOS and more than 1300 female controls [8]. The investigators demonstrated that a variant within the FTO gene (rs9939609, which has been shown to predispose to common obesity) was significantly associated with susceptibility to the development of PCOS.

Wickenheisser *et al* reported that CYP17 promoter activity was 4-fold greater in cells of patients with PCOS. This research suggests that the pathogenesis of PCOS may be in part related to the gene regulation of CYP17 [9]. However, in a study that assessed candidate genes for PCOS using microsatellite markers to look for association in 4 genes—CYP19, CYP17, FST, and INSR —only 1 marker near the INSR gene was found to be significantly associated with PCOS. The authors concluded that a susceptibility locus for PCOS (designated PCOS1) exists in 19p13.3 in the INSR region, but it cannot be concluded that the INSR gene itself is responsible [10].

Subsequent studies have found additional associations, such as those of 15 regions in 11 genes previously described to influence insulin resistance, obesity, or type 2 diabetes [11]. Individuals with PCOS were found more likely to be homozygous for a variant upstream of the PON1 gene and homozygous for an allele of interest in IGF2. Interestingly, the PON1 gene variant resulted in decreased gene expression, which could increase oxidative stress. The exact result of the IGF2 variant is unclear, but IGF2 stimulates androgen secretion in the ovaries and adrenal glands [12].

In study by Goodarzi *et al*, the leucine allele was found to be associated with protection against PCOS, as compared to the valine allele at position 89 in SRD5A2. The leucine allele is associated with a lower enzyme activity [13]. When the results of this study are combined with those of an observational study by Vassiliadi *et al*, based on urinary steroid profiles in women with PCOS, further support can be found for an important role for 5-alpha reductase in the pathogenesis of this syndrome [14].

In a genome-wide association study for PCOS in a Han Chinese population, 3 strong regions of association were identified, at 2p16.3, 2p21, and 9q33.3 [26]. The polymorphism most strongly associated with PCOS at the 2p16 locus was near several genes involved in proper formation of the testis, as well as a gene that encodes a receptor for luteinizing hormone (LH) and human chorionic

gonadotropin (HCG). This polymorphism was also located 211kb upstream from the FSHR gene, which encodes the follicle-stimulating hormone (FSH) receptor [15].

The polymorphisms most strongly associated with PCOS at the 2q21 locus encode a number of genes, including the THADA gene, which has previously been associated with type 2 diabetes. In addition, 6 significant polymorphisms were identified as being associated with PCOS at the 9q33.3 locus near the DENND1A gene, which interacts with the ERAP1 gene. Elevation in serum ERAP1 has been previously associated with PCOS and obesity [16].

In the United States, polycystic ovarian syndrome (PCOS) is one of the most common endocrine disorders of reproductive-age women, with a prevalence of 4-12% [17, 18]. Up to 10% of women are diagnosed with PCOS during gynecologic visits [19]. In some European studies, the prevalence of PCOS has been reported to be 6.5-8%. [20]. A great deal of ethnic variability in hirsutism is observed. For example, Asian (East and Southeast Asia) women have less hirsutism than white women given the same serum androgen values. In a study that assessed hirsutism in southern Chinese women, investigators found a prevalence of 10.5%. In hirsute women, there was a significant increase in the incidence of acne, menstrual irregularities, polycystic ovaries, and acanthosis nigricans.

PCOS affects premenopausal women, and the age of onset is most often perimenarchal (before bone age reaches 16 y). However, clinical recognition of the syndrome may be delayed by failure of the patient to become concerned by irregular menses, hirsutism, or other symptoms or by the overlap of PCOS findings with normal physiologic maturation during the 2 years after menarche. In lean women with a genetic predisposition to PCOS, the syndrome may be unmasked when they subsequently gain weight. Evidence suggest that women with polycystic ovarian syndrome (PCOS) may be at increased risk for cardiovascular and cerebrovascular disease. Women with hyperandrogenism have elevated serum lipoprotein levels similar to those of men [21].

Approximately 40% of patients with PCOS have insulin resistance that is independent of body weight. These women are at increased risk for type 2 diabetes mellitus and consequent cardiovascular complications. The American Association of Clinical Endocrinologists and the American College of Endocrinology recommend screening for diabetes by age 30 years in all patients with PCOS, including obese and non-obese women. In patients at particularly elevated risk, testing before 30 years of age may be indicated. Patients who initially test negative for diabetes should be periodically reassessed throughout their lifetime.

Patients with PCOS are also at an increased risk for endometrial hyperplasia and carcinoma. The chronic anovulation in PCOS leads to constant endometrial stimulation with estrogen without progesterone, and this increases the risk of endometrial hyperplasia and carcinoma. The Royal College of Obstetricians and Gynaecologists (RCOG) recommends induction of withdrawal bleeding with progestogens a minimum of every 3-4 months. No known association with breast or ovarian cancer has been found; thus, no additional surveillance is needed.

The National Institute of Health (NIH) criteria include clinical and/or biochemical hyperandrogenism and chronic anovulation for diagnosing PCOS whereas the most recent androgen excess and PCOS (AE–PCOS) Society criteria recommend that PCOS should be defined as clinical or biochemical hyperandrogenism associated with ovulatory dysfunction in the form of oligo-anovulation or polycystic ovaries on ultrasound. Insulin resistance and hyperandrogenism is found to play a key role in the pathogenesis of polycystic ovarian syndrome. Hence based on above findings the present study was planned for Clinical Assessment of anovulatory infertile women with PCOS by Comparative Administration of N-Acetyl Cysteine with Metformin.

Methodology

The present study was planned in Department of Obstetrics & Gynaecology, Government Medical College Bettiah, West Champaran, Bihar, India. The 50 females diagnosed with the polycystic ovarian syndrome (PCOS) were enrolled in the present study. The 25 females were administered with the Metformin and 25 females were administered with the N- Acetyl cysteine (NAC).

All the patients were informed consents. The aim and the objective of the present study were conveyed to them. Approval of the institutional ethical committee was taken prior to conduct of this study.

Following was the inclusion and exclusion criteria for the present study.

Inclusion criteria: Anovulatory infertile with PCOS as per Rotterdam criteria.

Exclusion criteria: Hypersensitivity to metformin or NAC, pelvic organ pathologies, congenital adrenal hyperplasia, thyroid dysfunction, Cushing's syndrome, hyperprolactinemia, androgen-secreting neoplasia, diabetes mellitus, consumption of medication affecting carbohydrate metabolism within 3 month before the study, patients taking hormonal analogue other than progesterone, severe hepatic or renal disease and active peptic ulcer.

Results & Discussion

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) [22].

Metformin, an insulin sensitizing agent have been found to have a promising effect in improving the clinical, hormonal and biochemical profile of women with PCOS. The mechanism of action of the metformin is by improving insulin sensitivity thereby lowering insulin levels resulting

in increase in sex hormone binding globulin and hence decreases in androgens [23]. N-acetyl cysteine (NAC) a derivative of amino acid L-cysteine is an essential precursor used by the body to produce glutathione which is a powerful antioxidant and hence inhibit oxidative stress and consequently prevents hyperinsulinemia induced insulin resistance and preserves insulin receptors from oxidative stress [24].

Table 1: Basic Details

Group	Group I	Group II
Administration of	**Metformin**	**NAC**
No. of Cases	25	25
Socio Economic Class		
Upper	2	2
Middle	20	18
Lower	3	5
Body Mass Index kg/cm2	22.3 – 25.1	21.4 – 26.6

Table 2: Basic Details

Group	Group I		Group II	
Administration of	**Metformin**		**NAC**	
Treatment	Pre	Post	Pre	Post
Weight kg	54 – 72	53 – 69	55 – 65	52 – 72
Waist Circumference	85.4 – 95.3	84.2 – 94.5	87.2 – 96.7	84.5 – 90.1
waist-hip ratio	0.87 – 0.94	0.86 – 0.93	0.86 – 0.98	0.81 – 0.92
Fasting Glucose	89 – 115	76 – 98	95 – 115	72 – 96
Total testosterone	1.35 – 2.75	1.25 – 2.48	1.25 – 2.69	0.95 – 2.05

BMI was more marked in NAC group compared to metformin group and the difference was statistically significant (p=0.039). The decrease in BMI after treatment with NAC in present study is comparable to the study conducted by Salehpour S *et al* they observed a statistically significant decrease in BMI after treatment with NAC [25]. The effect of metformin and NAC on BMI in the present study was not concordant with the study conducted by Oner G *et al*. They did not find significant decrease in BMI in both metformin and NAC groups after six months of treatment [26]. Similar observation of no significant decrease in BMI was noted by Elnashar A *et al*. [27] Women with PCOS present with menstrual irregularity consequent to anovulation and increased androgen levels. Hyperinsulinemia leads to increased androgen production and abnormal LH and FSH secretion [28]. All these factors lead to oligo or anovulation with resultant menstrual irregularity.

Anovulation is a common cause of infertility in women with PCOS. Of all women with anovulatory infertility 75% are because of PCOS [29]. A significant correlation exists between insulin resistance and abnormal ovarian function in women with PCOS. It has been proposed that higher the insulin resistance in PCOS patients lower the probability of ovulation [30]. Thus any intervention aimed at lowering the insulin resistance is likely to improve ovulation in PCOS women.

Similarly in a study conducted by Gayatri *et al*. [31] found significant improvement in some of the clinical features like weight gain, BMI, WHR, acne and hirsutism in group N (P\0.05), but there was no significant change in other features like oligomenorrhea, amenorrhea and infertility. Salehpour *et al*. [32] conducted prospective experimental clinical trial of NAC with placebo in a group of 36 patients. There was a significant reduction of weight, BMI, WC and WHR in NAC group as compared with placebo.

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 [33] there occurs inhibition of oxidative stress and consequently the prevention of hyperinsulinemia induced insulin resistance and preservation of insulin receptors against oxidant agents [34]. Metformin, (molecular formula C4H11N5) 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 treatment 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.

For some time the attention of the recent scientific literature has shifted to a new molecule: the N-acetyl- cysteine (NAC). Recent data have shown that NAC, a mucolytic drug acting as insulin sensitizer, represents an effective and safe strategy in the treatment of PCOS patients [35, 36]. This molecule appears to exert its beneficial effect both by increasing the insulin secretion by the beta cells of the pancreas and by inducing an increased sensitivity to the organism itself. In particular, some data show that a moderate increase in plasma thiols improves the consumption of glucose during the hyperglycemic clamp [36]. Other studies suggest a role of NAC in the regulation of the insulin receptor in human erythrocytes [37]. It has been shown, in fact, that high doses of NAC increase the cellular levels of reduced glutathione (GSH), a powerful antioxidant that seems to exert a certain influence on activity of the insulin receptor in vivo [38].

The role of insulin resistance in menstrual dysfunction in women with PCOS is already well known and documented in the literature. The importance of insulin resistance in the pathogenesis of the menstrual cycle disorders and infertility is also suggested by the observation that the administration of insulin-sensitizing drugs, such as metformin, can induce an improvement in spontaneous ovulation, promotes efficiency of ovulation-inducers drugs, and increases pregnancy rate [35].

Conclusion

The data generated from the present study concludes that NAC improves the clinical features, biochemical markers of insulin resistance, hormonal levels, anovulation and consequently the long-term health status of women with PCOS through inhibition of oxidative stress and improvement of peripheral insulin more effectively when compared with metformin. Due to the lack of adverse effects, NAC can be regarded as an appropriate substitute for insulin-reducing medications in the treatment of PCOS patients.

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