

# Treatments in Patients with Polycystic Ovary Syndrome and Effects on Kisspeptin Serum Levels

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## ABSTRACT

**Introduction:** Polycystic ovarian syndrome (PCOS) is a common and multifactorial pathology among young women of reproductive age and one of the main causes for female infertility. Most patients are referred to a gynecologist due to specific symptoms such as menstrual irregularities, hirsutism, facial or body acne and inability to obtain a pregnancy. Lately, kisspeptin, a neuropeptide produced in the anterior region of the hypothalamus that seems to modulate the GnRH secretion and therefore the hypothalamic-hypophyseal-ovarian axis (HHO-axis), has been considered responsible for PCOS occurrence.

**Objectives:** The aim of our study was to observe the changes in hormonal balances and kisspeptin serum levels in patients with PCOS after treatment with myo-inositol and vitex.

**Materials and methods:** A prospective clinical study was conducted by us, between 4 January 2021 and 1 March 2022, in “Elena Doamna” Clinical Hospital of Obstetrics and Gynecology, Iasi, Romania. After obtaining the subjects’ informed consent and agreement to participate, we identified 14 patients with PCOS and primary or secondary infertility, who underwent laboratory investigations during the follicular phase of their menstrual cycles, testing for kisspeptin, luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol, testosterone, insulin, blood sugar levels and prolactin. An abdominal-pelvic scan and full body examination were also performed. Patients, in accordance with their gynecologists’ recommendation, preferred a medical therapy consisting of myo-inositol 750 mg/day and vitex 400 mg/day. After three months of treatment, patients agreed to have a second check up to see if any changes occurred.

**Results:** We found significant positive differences when considering kisspeptin, estradiol, FSH and prolactin serum values. There was no significant difference in testosterone and insulin serum levels; on the other hand, we observed an increase in LH serum levels. The mean ovarian volume before treatment was 14.0 for the right ovaries and 13.8 for the left ovaries. After treatment and the recommended lifestyle changes, we noticed a decrease in the mean ovarian volume (13.5 for the right ovaries and 13.4 for the left ovaries).

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**Discussions and conclusions:** *The etiology of PCOS, which is considered a multifactorial pathology, remains unclear. Multiple studies have been conducted and are still exploring the role of kisspeptin as an etiologic factor for PCOS associated with infertility. Our study showed a decrease in kisspeptin values after the use of myo-inositol and vitex in patients with PCOS, therefore supporting the long-standing theory that kisspeptin might have an essential role in the development of PCOS.*

**Keywords:** kisspeptin, PCOS, myo-inositol, vitex.

## INTRODUCTION

Polycystic ovarian syndrome (PCOS) represents a multifactorial pathology that mainly affects women of reproductive ages. Its etiology is complex and still incompletely understood, with genetic, environmental, and lifestyle factors remaining among the most frequently incriminated determinants. Women suffering from PCOS are often examined for infertility issues, hyperandrogenism, dysfunction of menstrual cycles, acne, hirsutism, obesity and insulin resistance (1). Metabolic syndrome features, including dyslipidemia, hepatic steatosis, cardiac complications and diabetes mellitus, are also encountered in female patients diagnosed with PCOS (2). Rotterdam criteria allow physicians to easily diagnose patients with PCOS and require the presence of at least two of the three following well-known criteria: oligo-anovulation, biological or clinical signs of hyperandrogenism, and micropolycystic ovary syndrome (ovarian volume >10 mL and/or more than 12 ovarian follicles) (3). More than 40% of female infertility is linked to PCOS (4). Polycystic ovarian syndrome can affect women from the time of puberty through the reproductive years to menopause, with certain particularities for each life stage. In adolescence, the diagnosis is difficult due to the normal pubertal cycle that can be associated with irregular and anovulatory menstrual cycles; however, if an adolescent girl has oligo-amenorrhic cycles at the age of 15, she will show such symptoms in her adult life as well (5). Patients with PCOS signs are included in a specific class of “high risk for PCOS”, and these girls need a pre-cautious follow-up for up to eight years since menarche (6). Studies report that menopause occurs later in these types of patients and they tend to have regular ovulatory cycles at the end of their reproductive years (7).

When considering the reproductive age, anovulatory subfertility is identified in almost 70% of PCOS patients (8). Hormonal treatments were reported more often in patients with PCOS (62%) than those without it (33%). Various meta-analysis studies reported pregnancy and delivery complications in PCOS patients, including gestational diabetes, gestational hypertension, preeclampsia and higher rates for cesarean section (9).

Evaluation of infertility (or subfertility) must be performed after 12 months of trying to obtain a pregnancy, with couples having regular sexual intercourse but without results. Polycystic ovarian syndrome is one of the main causes for female infertility but the diagnosis should be implied only after excluding other endocrine pathologies such as thyroid dysfunctions, congenital adrenal hyperplasia, hyperprolactinemia, premature ovarian insufficiency or other anatomical dysfunctions and iatrogenic causes. The patient’s medical and surgical history should be obtained in detail, followed by a complete body check and gynecological examination and additional diagnostic tests. Blood samples, pelvic ultrasounds and hysteroscopy or laparoscopic procedures should be considered when investigating a female patient with infertility due to PCOS (10). Although the anti-mullerian hormone (AMH) is commonly tested in patients with infertility, in these cases its levels can be 2-3 times higher than normal values due to the increased number of preantral and small antral follicles.

Reproductive function, including ovulation, depends on a proper development and regulation of the hypothalamic-hypophyseal-ovarian (HHO) axis. A new gene, named KISS1, has been recently recognized as an important regulator of the hypothalamic-pituitary-gonadal (HPG) axis, which is responsible for the normal onset of puberty, normal cyclical function and ovulation during adult life. Kisspeptins is expressed in the human brain and represents a family of neuropeptides enco-

ded by KISS1 that acts through its main receptor G-protein-coupled receptor (GPR54) (12). Kisspeptin connects to GPR54 receptors found in the gonadotropin-releasing hormone (GnRH) neurons and regulates the HHO axis (13). Through the activation of GnRH neurons, kisspeptin stimulates the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the gonadotrope cells in the anterior region of the pituitary gland (14). Given the dysregulated gonadotropin secretion in PCOS and the essential role of kisspeptin as a key regulator of GnRH secretion, it is easy to hypothesize that PCOS is actually a reflection of altered kisspeptin secretion.

Considering the multifactorial origin of PCOS, the appropriate treatment involves different non-pharmacological and pharmacological measures. The first line of treatment is represented by non-pharmacological interventions, which are shown to be effective in patients with PCOS suffering from infertility. One of the newest therapeutic approaches is represented by the administration of inositol compounds. In all smoker patients, cessation of tobacco consumption is recommended, as well as losing weight in those whose body mass index (BMI) is above normal values (15). Weight loss also improves the effect of ovulation-inducing therapies and is shown to improve the rates of pregnancy achievement (16).

Inositol represents one of the newest and most recently investigated therapies in patients with infertility due to PCOS. Currently, only nine different stereoisomers of inositol have been identified, of which myo-inositol was shown to be the most widely encountered and easy to find in both vegetal and animal nutrients. D-chiro-inositol is the second most commonly occurring isomer (17). Inositol isomers are directly involved in insulin cellular signaling and can be formed from glucose in human cells (18). They act as intracellular second messengers that regulate hormones such as thyroid-stimulant hormone (TSH), FSH and insulin. Recent studies have shown that D-chiro-inositol was effective in reducing insulin resistance and improving the metabolic syndrome in patients with PCOS. Based on existing theory, 1 g of D-chiro-inositol and 400 mcg of folic acid *per day* represent the required dose to reduce metabolic syndrome effects. Worldwide, inositol has been introduced in IVF protocols and studies that aim to show its beneficial action are conducted (19). □

## MATERIALS AND METHODS

We conducted our study in “Elena Doamna” Clinical Hospital of Obstetrics and Gynecology, Iasi, Romania, between 4 January 2021 and 1 March 2022. It was a prospective case-control study, which included patients aged 18-45 years with normal BMI ranging between 18.5 and 30 kg/m<sup>2</sup>. Patients were diagnosed with PCOS according to Rotterdam criteria prior to or on commencement of study and all of them had primary or secondary infertility. Patients with associated endocrine pathologies that might have interfered with our hormonal laboratory exams or clinical examination were excluded. All patients agreed to participate in our study and signed a consent form before being examined. The following exclusion criteria were used: patients aged under 18 or above 45 years, those who either had abnormal BMI values, endometriosis, autoimmune diseases, prolactinomas, thyroid pathologies, Cushing’s disease or other potential causes for anovulation and/or hyperandrogenemia, or were using a hormonal treatment.

All patients signed their informed consent and fully agreed to participate in our study. Clinical examination included weight, height and body mass index measurements; also, all signs related to PCOS such as acanthosis nigricans, menstrual cycle disturbances, facial or body acne and hirsutism were noted in each patient’s medical chart. Menstrual irregularities included amenorrhea explained by the absence of menstrual cycles for more than six months and oligomenorrhea marked by delayed menstrual cycles for more than 35 days over a six-month period.

Anamnesis and the patient’s medical history were noted in detail and recorded for the study as well. Blood samples were collected from all patients during the follicular phase of their menstrual cycle and an abdominal-pelvic scan was performed. Laboratory exams tested the serum levels for kisspeptin, LH, FSH, estradiol, insulin, glycemic levels, testosterone and prolactin. Five milliliters of blood were collected from each patient. After applying the exclusion and inclusion criteria, 14 patients diagnosed with PCOS and primary or secondary infertility were finally included in our study.

Patients received first-line treatment with myo-inositol and vitex for three months from their ge-

neral practitioners. They were all informed about healthy lifestyle behaviors such as reducing or quitting tobacco use, engaging in regular physical activity for at least 30 minutes five days a week, avoiding foods high in estrogen and factors that might increase mental or physical stress.

Patients accepted a new appointment for a second blood sampling three months after treatment with myo-inositol (one capsule of 750 mg per day) and vitex (one capsule of 400 mg per day) and lifestyle changes recommended by their general practitioners. All underwent hormone testing again as well as clinical examination consisting of weight, height and BMI measurements in order to identify any positive or negative changes. All signs of acanthosis nigricans, facial and body hirsutism and acne were assessed after treatment. □

## RESULTS

After obtaining pre- and post-treatment laboratory test results, we identified slightly positive changes in the hormonal balance and physical features of patients with PCOS.

We noticed significant positive differences when considering kisspeptin, estradiol, FSH and prolactin serum values. There was no significant difference in testosterone and insulin serum levels but an increase in LH serum levels was observed.

The high kisspeptin values, which were considered a new mark for PCOS, decreased by approximately 25.8%, estradiol levels decreased by 39.4% and FSH levels were almost four times higher than their values before treatment. Prolactin serum levels decreased by 43.2%. There was

no significant differences in testosterone and insulin levels. Contrary to our expectations, LH serum levels increased by 35.9% (Table 1).

The mean ovarian volume before treatment was 14.0 for the right ovaries and 13.8 for the left ovaries. After treatment and lifestyle changes, we identified a decrease in the mean ovarian volume by 13.5 for the right ovaries and 13.4 for the left ovaries.

When we asked our patients about the perceived benefits during the treatment, 80% of them reported an obvious improvement in their symptoms, including regulation of menstruation, decrease in hirsutism and facial and body acne, and a better mental health state. Their BMI remained within normal limits as they were before treatment and no significant change was noticed. However, most patients acknowledged a substantial improvement in their quality of life after combining medication with lifestyle changes (mainly physical activity and mental stress reduction).

Half of our patients decided to continue medical therapy with inositol and vitex on long term and in accordance with their gynecologists. Two patients managed to obtain pregnancies after long term treatment but both ended in miscarriages during the first six weeks of gestational age. □

## DISCUSSIONS

Polycystic ovarian syndrome is considered a multifactorial pathology and therefore, its etiology remains unclear (20). Multiple studies have been conducted and are still exploring the role of kisspeptin as an etiologic factor for PCOS associated with infertility. Studies performed by Chan *et al* (21), Yilmaz *et al* (22) and Jeon *et al* (23) have all reported significantly higher kisspeptin serum levels in patients with PCOS when compared to control groups. Our study showed a decrease in kisspeptin values after the use of myo-inositol and vitex in patients with PCOS, therefore supporting the long-standing theory that kisspeptin might have an essential role in the development of PCOS. Although it can be disputed that the relatively small number of patients may not be sufficient in order to create a strong and clear relationship between kisspeptin and PCOS, it surely represents a start-up and a theory that needs to be further confirmed within large studies. Our findings may also be influenced by

**TABLE 1.** Comparisons between hormonal levels and pelvic scan results before and after treatment with myo-inositol and vitex for three months

	Before treatment	After treatment
Mean kisspeptin serum levels	99,126 pg/mL	73,53 pg/mL
Mean estradiol serum values	61,626 pg/mL	37,323 pg/mL
Mean LH serum values	13,962 mUI/ml	18,98 mUI/mL
Mean FSH serum values	6,147 mUI/mL	25,735 mUI/mL
Mean testosterone values	0,662 ng/mL	0,649 ng/mL
Mean insulin values	12,000 $\mu$ UI/mL	12,200 $\mu$ UI/mL
Mean prolactin values	617,282 $\mu$ UI/mL	350,158 $\mu$ UI/mL

the dosage used for myo-inositol and vitex associated with lifestyle changes. Our patients took 750 mg of myo-inositol plus 400 mg of vitex daily for only three months, which was comparable to the doses of  $2 \times 2000$  mg myo-inositol and  $2 \times 200$  mcg folic acid *per day* for two and three months, which were administered in the study of Regidor *et al* (24); their results suggested that a myo-inositol therapeutic management in PCOS patients induced a better fertilization rate and better embryo quality. When used in *in-vitro*-fertilization (IVF) protocols, the dosage of myo-inositol recommended is 4000 mg *per day* (25). In the review of Pundir *et al* (26), the authors reported that supplementation with inositol appeared to increase the rates of ovulation and the regulation of menstrual cycles. No study identified a better live birth rate. In a study published in 2017, Legro *et al* explored alternate and future paths in the treatment of PCOS and proved that myo-inositol and D-chiro-inositol taken alone or in combination led to an increase

in the frequency of ovulation (27). When dealing with infertile women and therapeutic supplementation, it is indicated to consider the positive effects and benefits of vitamin D, alfa-lipoic acid, folic acid and omega-3 as well (28).  $\square$

## CONCLUSION

When considering our results after only three months of treatment, including the decrease in kisspeptin serum levels associated with reduction in mean right and left ovarian volume and quality of life improvement, we imply the theory that myo-inositol most surely has a positive effect on patients with PCOS and infertility. Our study is the first of its kind to be performed on the general population in Romania and we believe it is as a new start for updating new and innovative treatment in PCOS.  $\square$

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