A CROSS SECTIONAL STUDY ON THE STATUS OF INFLAMMATORY MARKERS IN POLYCYSTIC OVARY SYNDROME (PCOS) PATIENTS IN INDIAN POPULATION

DISSERTATION

SUBMITTED FOR
M.D. IN PHARMACOLOGY

THE TAMILNADU Dr. M.G.R MEDICAL UNIVERSITY

DEPARTMENT OF PHARMACOLOGY
PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH
PEELAMEDU, COIMBATORE- 641 004
TAMILNADU, INDIA

APRIL 2015
This is to certify that this dissertation entitled “A CROSS SECTIONAL STUDY ON THE STATUS OF INFLAMMATORY MARKERS IN POLYCYSTIC OVARY SYNDROME (PCOS) PATIENTS IN INDIAN POPULATION”, is a work done by Dr. R. SENTHURSELVI, Postgraduate under the guidance of Dr. K. BHUVANESWARI, M.D., Professor and Head, Department of Pharmacology, PSG IMS&R.

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DECLARATION

I solemnly declare that the dissertation titled “A CROSS SECTIONAL STUDY ON THE STATUS OF INFLAMMATORY MARKERS IN POLYCYSTIC OVARY SYNDROME (PCOS) PATIENTS IN INDIAN POPULATION” was done by me under the guidance and supervision of Dr. K. BHUVANESWARI, M.D.,

This dissertation is submitted to the Tamilnadu Dr.MGR Medical University towards the partial fulfillment of the requirement for the award of M.D Degree in Pharmacology.

Place: Dr. R. SENTHURSELVI

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Ref.: Proposal titled: "A Cross-sectional study on the status of inflammatory markers in Polycystic Ovary Syndrome (PCOS) patients in Indian population"

Sub.: Ethics Committee Approval for the study

The Institutional Human Ethics Committee, PSG IMS, Coimbatore 641 004, has reviewed your proposal on 25th April, 2014 in its full board review meeting held at College Council Room, PSG IMS&RI, between 2.00 pm and 3.45 pm, and discussed your application to conduct the study entitled:

"A Cross-sectional study on the status of inflammatory markers in Polycystic Ovary Syndrome (PCOS) patients in Indian population"

The following documents were received for review:

1. Duty filled application form
2. Proposal (Ver 1.1)
3. Informed Consent forms
4. Data collection tool
5. Budget
6. CV

The members who attended the meeting at which your study proposal was discussed are as follows:

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<th>Sl. No.</th>
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<th>Qualification</th>
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After due consideration, the committee has decided to approve the above proposal.

The approval is valid for one year.

We hereby confirm that neither you nor any of your study team members have participated in the voting/decision making procedure of the committee. The members of the committee who have participated in the voting/decision making procedure of the committee do not have any conflict of interest in the referenced study.

This Ethics Committee is organized and operates according to Good Clinical Practice and Schedule Y requirements.

Non-adherence to the Standard Operating Procedures (SOP) of the Institutional Human Ethics Committee (IHEC) and national and international ethical guidelines shall result in withdrawal of approval (suspension or termination of the study). SOP will be revised from time to time and revisions are applicable prospectively to ongoing studies approved prior to such revisions.

PIs are required to send progress reports (in the form of an extended abstract with publications if any) to the IHEC every six months (and a month before expiry of approval date, if renewal of approval is being sought).

Request for renewal must be made at least a month ahead of the expiry of validity along with a copy of the progress report.

PSG INMAS
COIMBATORE-641004

Dr S Bhuvaneswar
Member-Secretary
Institutional Human Ethics Committee
A CROSS SECTIONAL STUDY ON THE STATUS OF INFLAMMATORY MARKERS IN POLYCYSTIC OVARY SYNDROME (PCOS) PATIENTS INDIAN POPULATION

INTRODUCTION:

In prehistoric times (pre-1700) the average life expectancy was twenty years. This was because of increased mortality during childhood. If one survives more than five years then it is likely that he will reach adulthood. During 1500-1600 it was 35-40 years. This wide variation in mortality was due to war, famines and epidemics. All our ancestors were living a healthy life. The environment was not polluted and they had good
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A CROSS SECTIONAL STUDY ON THE STATUS OF INFLAMMATORY MARKERS IN PROGRESSIVE SYMPTOM SYNDROME (PSS) PATIENTS INDIAN POPULATION

INTRODUCTION

In previous times (pre 1780), the average life expectancy was twenty years. The increase of medical awareness during adulthood led to an increase of chronic diseases. This increase in chronic diseases led to a decrease in overall life expectancy. Chronic diseases such as diabetes and cardiovascular diseases, which are global leading cause of death, are more prevalent in developed countries. In contrast, in developing countries, acute infections are more prevalent. In both cases, the leading cause of death is due to chronic diseases.

In India, diabetes is the leading cause of death. In India, diabetes is the leading cause of death. In contrast, in developing countries, acute infections are more prevalent. In both cases, the leading cause of death is due to chronic diseases.

In this study, we will evaluate the role of diabetes in the mortality rate and the impact of diabetes on the overall life expectancy. In the Indian setting, the prevalence of diabetes is increasing, and the prevalence of diabetes is increasing, and the prevalence of diabetes is increasing. In this study, we will evaluate the role of diabetes in the mortality rate and the impact of diabetes on the overall life expectancy.

In the case of diabetes, the prevalence is increasing, and the prevalence is increasing, and the prevalence is increasing. In this study, we will evaluate the role of diabetes in the mortality rate and the impact of diabetes on the overall life expectancy. In the case of diabetes, the prevalence is increasing, and the prevalence is increasing, and the prevalence is increasing. In this study, we will evaluate the role of diabetes in the mortality rate and the impact of diabetes on the overall life expectancy.
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A CROSS SECTIONAL STUDY ON THE STATUS OF INFLAMMATORY MARKERS IN POLYCYSTIC OVARY SYNDROME (PCOS) PATIENTS IN INDIAN POPULATION

ABSTRACT:
AIMS AND OBJECTIVES: To assess the status of inflammation in patients with Polycystic Ovary Syndrome (PCOS) and to assess the influence of drugs prescribed for PCOS on inflammation.

MATERIALS AND METHODS: Patients were divided into three groups as healthy volunteers, newly diagnosed PCOS patients, already on treatment patients for PCOS. hs-CRP, IL-6 and IL-18 were assessed.

RESULTS: There was an increase in the hs-CRP, IL-6 and IL-18 compared to healthy volunteers in both newly diagnosed and already diagnosed groups. But there was no statistical significance (hs-CRP=0.975, IL-6=0.746, IL-18 = 0.148) between them. The mean values has decreased in the already on treatment group as duration of treatment increases. There was no statistical significance between them.

CONCLUSIONS: There was an inversely proportional relationship between the mean values of inflammatory markers and duration of treatment.
INTRODUCTION:

In prehistoric times [pre-1700] the average life expectancy was twenty years. This was because of increased mortality during childhood. If one survives more than five years then it is likely that he will reach adulthood. During 1500-1600 it was 35-40 years. This wide variation in mortality was due to war, famines and epidemics. All our ancestors were living a healthy life. The environment was not polluted and they had good physical activity. Their food was free of pesticides.

In today’s modern world we follow all hygienic practices right from our home. By following all these practices we are able to bring down the mortality rate and increase the life expectancy. On the other hand in this modern world physical activity is reduced and all edible products we consume are treated with pesticides. We also lead a stressful life. All these leads to increased morbidity, which is what we pay to this modern world for increasing our life expectancy. Both men and women are equally affected and some disease are specific for either men or women. One among them is Polycystic Ovarian Syndrome (PCOS) which is seen exclusively in women of reproductive age group.
World Health Organization reports that Polycystic Ovarian Syndrome approximately affects 5-10% of the reproductive female population worldwide. A study by Ridhi R et al. shows it affects the Indian female adolescents approximately by 9.13%².

Women with PCOS have chronic inflammation. This in turn leads to increased incidence of non-communicable diseases like cardiovascular disease and diabetes³. **Chronic inflammation** in PCOS patients can be identified using serum markers such as C-reactive protein [CRP], Interleukin-6[IL-6] and Interleukin-18 [IL-18].

We did a thorough search of literature in subscribed journals of our institute (print and online), PubMed, Ovid, Google, etc., to look for the status of inflammatory markers in Polycystic Ovary Syndrome patients. Almost all the publications are from the western world (Europe). There was only one study from India on the women with Polycystic Ovary Syndrome showing the status of Inflammation mainly the atherosclerotic markers³. All PCOS patients undergo routine investigations like Ultrasound abdomen and blood investigations.

This study was basically carried out to see if there is any association between inflammation and PCOS. If the serum inflammatory markers are higher, then like western population, there is an association between
inflammation and PCOS in Indian Population. We would also like to see
drugs used in PCOS has any influence over the inflammation in PCOS.
AIMS & OBJECTIVES:

1. To assess the status of inflammation in patients with Polycystic Ovary Syndrome (PCOS)

2. To assess the influence of drugs prescribed for PCOS on inflammation
PCOS is a disorder with varied presentation which form a wide range of signs and symptoms. The presentation may be mild in some and severe in others with respect to reproductive, endocrine and metabolic functions. The prevalence of polycystic ovaries in general population is 20-33% detected using ultrasound or other pelvic imaging modalities. The first histological description of polycystic ovary and features of the condition was made by Vallisneri in the year 1721. In 1935, it was Stein Sr. and Leventhal, two American Gynecologists saw presence of ovarian cysts along with anovulation. They described it as Polycysic Ovary Syndrome (PCOS). Initially presence of ovarian cysts with anovulation was the investigative measures of the disease. Anovulation and increased levels of androgens but not the ovarian cysts were made the diagnostic criteria later. The histology of polycystic ovary was of an ovary with prominent theca, fibrotic thickening of the tunica albuginea and multiple cystic follicles. For many years wedge resection was the only treatment for PCOS and histological assessment of the ovaries was therefore routine practise. The histopathological criteria have been defined as the observation of: atretic follicle and/or degenerating
granulosa cell, hypertrophy and leutenisation of the inner theca cell layer, and thickened ovarian tunica\textsuperscript{6}.

Ultrasound came into the picture during early 1980’s by using high-resolution real-time sector scanners\textsuperscript{7,8}. Using ultrasound ovarian appearance was described in women classified as having PCOS (by symptoms and serum endocrinology). Orsini et al described ovaries as either being predominantly solid if fewer than four small (<9mm) cystic structures were detected in the ovary or predominantly cystic if multiple cystic structures or at least one large cyst (>10mm) were present\textsuperscript{9}. Adams et al. defined a polycystic ovary as in one plane, at least 10 follicles (usually between 2 and 8mm in diameter) arranged peripherally around a thick core of ovarian stroma or dispersed all over in an increased amount of stroma according to an abdominal ultrasound\textsuperscript{10}. When scattered throughout the stroma the cysts were usually between 2-4mm in diameter\textsuperscript{11}. The criteria of Adams and colleagues have been implemented by various subsequent reports which used this criteria to identify polycystic ovaries\textsuperscript{3, 12, 13-18}. Abder Gadir et al found that the visualization of the polycystic ovaries supported the diagnosis of the syndrome in women with signs and symptoms, rather than being key in making the diagnosis\textsuperscript{16}. 
After examining tissues of ovary at hysterectomy or wedge resection it was found to have a good correlation between ultrasound diagnosis of polycystic morphology and histopathological criteria for polycystic ovaries $^{19,20}$.

It was recognized that PCOS is a heterogeneous condition and several large series exist that describe populations of patients with the condition. The components that define PCOS should include no less than two of the following: menstrual disturbance (oligo-anovulation), hyperandrogenism and polycystic ovaries. The first largest series of polycystic ovaries women was reported by Balen et al who discovered this spectrum of the disorder in 1741 patients$^{21}$. 38% of the women were overweight. Obesity was related with increased possibility of hirsutism, menstrual cycle abnormality and increased rate of infertility. The frequency of obesity was reliably large in most of the reports and most of them with polycystic ovaries were found to have menstrual cycle abnormalities than thin women with polycystic ovaries$^{21,22}$. Conway et al proved mean ovarian volume was higher in polycystic ovaries women who are diagnosed through ultrasound than the control group$^{23}$. Franks et al established a higher area of uterus and mean ovarian volume was doubled in the PCOS group$^{22}$. High serum LH (luteinizing hormone)
concentration were linked with infertility or menstrual cycle irregularities in both Frank and Balen study. Balen et al also identified that elevated levels of serum testosterone were related to increased potential of hirsutism, infertility and menstrual abnormalities. Ovarian volume strongly associated with serum levels of LH and testosterone concentration.

The incidence of PCOS are highly related to the assessing population’s nature. A cross-sectional study by Knochenhauer et al examined the prevalence of PCOS in a population of American women to be 4%\(^{24}\). There is ethnic variation in the prevalence of PCOS. The insulin resistance (IR) and symptoms were more in anovular South Asians than Caucasians with PCOS\(^{25}\).

In PCOS patients ovary is the primary site of endocrine abnormality particularly hyperandrogenism. It was Rosenfield et al who mentioned that disorder of P450c17\(\alpha\) plays a vital role in overproduction of androgen in ovaries\(^{26}\). It was later proved by others by injecting one dose of gonadotrophin releasing hormone agonist (GnRHa), naferalin to hyperandrogenic PCOS women in whom secretion of adrenal androgen had been inhibited by giving dexamethasone and their pituitary and ovarian response was found\(^{27}\). Franks et al extended this study to
anovulatory and ovulating hyperandrogenemic women and reported a little but substantial rise in androstenedione levels in both groups in reaction to GnRHa and an alike response in 17-hydroxyprogesterone levels, which were considerably higher in the women with anovulation. The elevation of these two hormones was not present when ACTH injection was administered. These results shows that hyperandrogenemia in both ovulatory and anovulatory women with PCOS is mostly of ovarian origin.

The distinctive morphology of ovaries is pathognomonic of this disease, its chief marker being hyperandrogenemia rising from the theca cells. Development of follicles is concerned with antral follicles detained at a diameter of 2-9mm. Study of follicle densities showed that normal ovaries had 11.4 small preantral follicles/m$^3$ (4-34), ovulatory polycystic ovaries had a density of 27.4 follicles/m$^3$ (9-81), and anovulatory polycystic ovaries had a density of 73.0 follicles/m$^3$ (31-94). This significant difference was also demonstrated for the primary follicles.

Serum LH concentrations are considerably higher in PCOS women than the controls. This is because amplitude and frequency of LH pulses are elevated. LH concentrations are increased which are seen in approximately 40-60% of PCOS women. Raised serum LH
concentrations has been related to decreased chance of conception and high risk of miscarriage\textsuperscript{35}. LH levels are predisposed by the temporal relation to ovulation, which quickly normalizes LH due to the suppressive effect of progesterone.

The pituitary gonadotropin is foremost to the function of reproduction. Its production and secretion of LH and FSH is directly stimulated by hypothalamic GnRH and is also affected by integrated feedback mechanism. FSH affords the early spur for follicular development and also helps alteration of androgens to estrogens in granulosa cells by stimulating the aromatase enzymes. LH plays a part in luteal phase by stimulating secretion of progesterone, also has a central role in the follicular phase, persuading thecal androgen production (substrate for estrogen synthesis) and initiating oocyte maturation at mid-cycle.

A single hypothalamic decapeptide, GnRH stimulates the gonadotroph to release both LH and FSH from the gonadotroph\textsuperscript{36}. Pulsatile GnRH stimulation is mandatory to maintain gonadotroph secretion. When GnRH pulsatility is sluggish FSH secretion outweighs, and when rapid LH secretion dominates\textsuperscript{37}. 


There are various causes that impact GnRH activity which comprise β-endorphin, estradiol (E₂), angiotensin II, melatonin, serotonin, neuropeptide-Y, oxytocin, neurotensin, somatostatin, dopamine and substance-P.
The increasing prevalence of obesity in western society should increase our awareness of the possibility of PCOS developing in genetically susceptible individuals. The association between obesity and raised free androgen levels is strong in women with increased central obesity.

**Picture 2: Functions of Leptin**

- **Hypothalamus**
  - ↓Neuropeptide-Y
  - ↑GnRH
  - ↑Sympathetic nervous system

- **White Fat**
  - ↓Food intake
  - ↑Thermogenesis
  - ↑Reproduction

- **Leptin**
  - ↑Insulin
  - Glucocorticoids
**Leptin** is a 167 aminoacid peptide that is produced by the fat cells in reaction to insulin and glucocorticoids. Leptin is conveyed by a protein which appears to be the extracellular domain of the leptin receptor itself. Leptin declines food intake and induces thermogenesis, it also seems to block the hypothalamic neuropeptide-Y, an inhibitor of GnRH pulsatility. Leptin appears to aid as the indicator from the body fat to the brain about the availability of fat stores for reproduction. Consequently menstruation will happen if stores of fat are sufficient. Obesity on the other hand is linked with elevated concentrations of leptin and this in turn could be a possibility for hyper secretion of LH in women with PCOS.

Many of the symptoms of PCOS are associated with psychological disturbance and any symptom may worsen a pre-existing tendency to psychological dysfunction. The menstrual cycle is commonly erratic in PCOS and periods when they occur are often heavy, prolonged and unpredictable. Furthermore in anovulatory women, estradiol acts on the endometrium without any opposition since there is absence of production of cyclical progesterone. This results in events of uneven menstruation, which can lead to endometrial hyperplasia and endometrial carcinoma.
Menstrual dysfunction brings with it various psychological problems, in particular, mood changes, which are often described as being similar to premenstrual type symptoms. It is likely that ‘hormonal imbalance’ or the erratic nature of the cycle may lead to altered mood and psychological distress\(^41\).

Insulin resistance and its associated hyperinsulinemia leads to a sequence of events collectively called as insulin resistant syndrome. The sequence of events include cardiovascular disease, hypertension, polycystic ovary syndrome and non-alcoholic steato hepatitis\(^42\). Insulin resistant syndrome and diabetes are inter-related but are different entities. All insulin resistant individuals need not be diabetic, but develops diabetes only if he is not able to make up the degree of hyperinsulinemia needed to overcome the insulin resistance. Eventhough an insulin resistant individual need not be diabetic the risk of developing the consequences of insulin resistance syndrome are high. Patients with insulin resistance syndrome have increased concentrations of plasminogen activator inhibitor-1, C-reactive protein and increased white cell count. Hence these patients are at increased risk of developing PCOS.
Insulin Resistance

Inadequate Insulin Response

Type 2 Diabetes

Retinopathy
Nephropathy
Neuropathy

Compensatory Hyperinsulinemia

Insulin Resistance Syndrome

Hypertension
PCOS
NAFLD

CVD
**Picture 4: Insulin Signaling Pathway**

**Insulin Receptor**

**ATP**

**IRS-1**

**IRS-2**

**IRS – Insulin Receptor Substrate**

**Tyr** - Tyrosine

**Ser** - Serine
Receptor of human insulin is a **heterodimer** consists of two α, β-dimers. A disulfide bond links both these α, β-dimers. The α-subunit is extracellular and comprises the binding domain for receptor. The β-subunit is intracellular and it contains the intrinsic protein tyrosine kinase activity. When a ligand binds to receptor of insulin it causes conformational change i.e autophosphorylation of this receptor at tyrosine site and promote stimulation of its intrinsic kinase activity. In PCOS patients there is fall in insulin receptor autophosphorylation. This is because of increased basal autophosphorylation. Phosphoaminoacid analysis demonstrated insulin-dependent receptor tyrosine phosphorylation was reduced and augmented insulin independent receptor serine phosphorylation. Serine phosphorylation of this receptor in initial phases of the insulin signaling pathway causes IR in PCOS women. Insulin independent serine phosphorylation of the insulin receptor in PCOS women is a sole condition of insulin action as further insulin-resistant states like obesity and non-insulin dependent diabetes mellitus [NIDDM] do not exhibit this abnormality.

**Low grade inflammation** which is of chronic is related to obesity, IR syndromes such as polycystic ovary syndrome, and an increased risk of cardiovascular disease. In obese patients enlargement of adipose tissue
causes adipocyte hypertrophy and hyperplasia and this huge adipocytes outshine the local supply of oxygen causing the cell hypoxia independently with stimulation of cellular stress pathway\textsuperscript{46}. This leads to independent inflammation of the cells and cytokines and other pro-inflammatory signals to get released. As a fragment of this chronic inflammatory process, locally released chemokines draws pro-inflammatory macrophages into the adipose tissue. These then produce structures similar to crown round the huge lifeless or dying adipocytes. These tissue macrophages release cytokines which then trigger the inflammatory process in adjacent adipocytes, aggravating inflammation and insulin resistance. There is genetic basis for the inflammation observed in PCOS\textsuperscript{47}. Variants in genes encoding several pro-inflammatory cytokines and their receptors associated with insulin resistance, obesity and/or diabetes have also been found to be associated with PCOS\textsuperscript{48-54}. Variants in the gene encoding TNF\textalpha\textsuperscript{49}, type 2 TNF receptor and IL-6\textsuperscript{51-53} and its signal transducer\textsuperscript{54} have been reported in association with PCOS in European population. These findings are in conceptual agreement with a common evolutionary background for PCOS and metabolic disorders.
There is controversy regarding relevance of circulating inflammatory molecules because most pro-inflammatory mediators exert their effect in tissue in autocrine and paracrine fashion. Interleukin-6 is an endocrine cytokine produced by mononuclear cell and adipose tissue that is directly responsible for stimulating hepatic CRP synthesis \(^{55-58}\). CRP in turn has emerged as a major predictor of metabolic dysfunction in asymptomatic individuals, and is also produced by adipose tissue \(^{59, 60}\). Hence **C-reactive protein** is the most consistent circulating marker of low-grade chronic inflammation in PCOS.

Like C-reactive protein and IL-6, IL-18 is also a proinflammatory cytokine which promotes secretion of TNF-\(\alpha\) \(^{61}\), which further stimulates the production of IL-6. **Interleukin-6** controls the production of CRP in the liver. Serum **Interleukin-18** levels also correlate with waist-to-hip ratio [WHR] and fasting insulin levels. Hence raise in serum IL-18 is related to obesity and insulin resistance. Monocyte chemoattractant protein-1 [MCP-1] and Macrophage inflammatory protein-1 [MIP-1] are other proinflammatory chemokines elevated in polycystic ovary syndrome. Monocyte chemoattractant protein plays a major role in the development of atherosclerosis, while Macrophage inflammatory protein is also called as chemokine [c-c motif] ligand 3 [CCL-3] which
shows a significant role in recruitment and activation of leukocytes and predicts future cardiovascular events\textsuperscript{62}.

In addition to pro-inflammatory cytokines and chemokines there are also other markers of inflammation found to be elevated in women with polycystic ovarian syndrome. These include the WBCs, oxidative stress, advanced stress, advanced glycation end products, endothelial inflammation and infections.

The WBC[White blood cell] count is found to be elevated in polycystic ovarian syndrome women with significant increase in lymphocytes and monocytes. Elevation of WBC count in women with polycystic ovarian syndrome correlated with the insulin resistance.

Oxidative stress and inflammation are inter-related. Hence there is an association between oxidative stress and diabetes, obesity, metabolic syndrome and polycystic ovarian syndrome. Oxidative stress is identified by evaluating lipid peroxidation using erythrocyte malondialdehyde assay. In patients with polycystic ovarian syndrome there is increase in lipid peroxidation and this was directly proportionate to body mass index [BMI], insulin levels and blood pressure. In addition patients with polycystic ovarian syndrome also have decreased total antioxidant status, reduced haptoglobin a protein with antioxidant
properties. Hence oxidative stress is directly proportional to insulin resistance and reactive oxygen species production by mononuclear cells in regard to hyperglycemia which is higher in women with polycystic ovarian syndrome.

When there is a non-enzymatic reaction between reducing sugar and amino groups of proteins advanced glycation end products [AGEs] are generated. There is induction of oxidative stress when these advanced glycation end products act on signal transducing receptors [RAGE]. Recent studies demonstrate an elevation of AGE products in women with polycystic ovarian syndrome\textsuperscript{63}.

Chronic low grade inflammation is linked to endothelial inflammation and subsequently to endothelial dysfunction. Polycystic ovarian patients have endothelial dysfunction this in turn is resistant to insulin induced vasodilation. Also polycystic ovary syndrome is linked with various markers of dysfunction of endothelium and inflammation including endothelin-1, soluble intercellular adhesion molecule-1 [sICAM-1], soluble vascular cell adhesion molecule-1 [sVCAM-1] and plasminogen activator inhibitor-1. Hence abnormal endothelial function in polycystic ovary syndrome is proportional to its insulin resistance.
There are few studies stating that there is an association between polycystic ovary syndrome and low grade chronic infections. Patients with oligomenorrhea and hirsuitism show seropositivity to Chlamydia Pneumonia and Chlamydia trachomatis\(^6\). These patients also have elevated high sensitive- C reactive protein. H.Pylori infection was also more prevalent in polycystic ovary syndrome women than age matched controls\(^6\).

The health magnitudes of this syndrome are a lifelong issue. The prevalence of impairment of glucose tolerance, insulin resistance and diabetes is raised considerably in polycystic ovary syndrome. Cardiovascular risk factors comprising high cholesterol levels, hyperandrogenemia, hypertension, markers of a prothrombotic state and inflammatory markers are increased in women with polycystic ovary syndrome. Altered vascular, endothelial function and increased incidence of metabolic syndrome are also noted in polycystic ovary syndrome.

Numerous therapies have been projected for the treatment modalities of polycystic ovarian syndrome, but the ideal treatment for infertile women with polycystic ovarian syndrome has not yet been clear. Drug treatment comprises clomiphene citrate, insulin sensitizing agents, gonadotropins
and gonadotropin-releasing hormone (GnRH) analogs. Surgical
treatments include laparoscopic ovarian drilling and the application of
assisted reproduction techniques. Besides this loss of weight, diet and
physical exercise have been shown to develop hyper insulinemia,
menstruation abnormalities and ovulatory rates.

Polycystic ovary syndrome women develop menstruation irregularities
owing to abnormal hormonal concentrations which in turn lead to the
development of endometrial carcinoma, so in a women with menstrual
irregularities it is more important to prevent development of endometrial
hyperplasia than to regularize her menstrual cycles. Hence intermittent
induction of menstruation using progestogens or intake of oral
contraceptives either cyclically or continuously inhibits atypical
proliferation of uterus. Combined oral contraceptives is utmost common
treatment for the symptoms of polycystic ovary syndrome as they
impede with actions of androgen and reduce androgen production,
increased hepatic serum hepatocyte binding globulin synthesis, binding
to androgen receptors competitively by some progestogens. Combined
oral contraceptives have been revealed to decline insulin sensitivity,
impair glucose tolerance and alter the lipid profile. Hence use of
combined oral contraceptives alone for polycystic ovary syndrome make
them vulnerable to metabolic distresses. Treatment that combine the combined oral contraceptives with insulin sensitzers, antiandrogens, or both are developing with possible favorable effects on metabolic defects, particularly young females with polycystic ovary syndrome.

**Metformin** is a biguanide and is connected to guanidine and galegine. It is extracted from the plant Galega officinalis. It was in1922 metformin was first defined in works by Werner and Bell as a product in production of “N”, “N”- dimethyl guanidine\(^6\).
They found that dicyanodiamide was the precursor to guanidine derivatives. Dicyanodiamide when reacts with dimethyl ammonium chloride in acidic conditions for three to four hours, then dimethyldiguanide [i.e. metformin] is formed in good yields. Interest in metformin started in 1950s where metformin did not cause fall in the blood pressure and heart rate in animals not like related compounds. Garcia thought metformin to have bacteriostatic, antiviral, antimalarial, antipyretic and analgesic action. In 1954, Polish Pharmacologist Janusz Sipniewski in a series of articles was not able to demonstrate most of these effects, including lowered blood sugar\textsuperscript{67}.

Sterne was the first one who used metformin on humans for the treatment of diabetes. He published his results in 1957 and called the drug “Glucophage” [glucose eater]. Only in 1994 U.S food and drug administration [FDA] gave approval for the drug, even though it was approved in Canada in 1972\textsuperscript{67}.

Since Polycystic ovary syndrome is linked with insulin resistance, antidiabetic therapy has been prepared since late 1980s. It was in the University of The Andes where metformin use in polycystic ovary syndrome was first started. In United Kingdom, National Institute of Health and Clinical Excellence suggested in 2004 to give metformin for
women with polycystic ovary syndrome and body mass index above 25. This was prescribed for anovulation and infertility if further treatment options failed to yield the results. Metformin is not recommended as the first line of treatment for polycystic ovary syndrome by the United Kingdom and International Clinical Practice guidelines, except for women with glucose intolerance.

Metformin acts by decreasing hepatic glucose production and increasing hepatic glucose uptake. This has been implicated as a major contribution to glucose lowering effect. AMP-activated protein kinase (AMPK) affords a contender goal mediating useful metabolic possessions of metformin. AMPK has a wider part in metabolic processes which includes oxidation of fatty acid, glucose uptake by the muscle, glucose stimulated genes associated with hepatic lipogenesis. Thus metformin by activating AMPK controls type 2 diabetes mellitus.

Various dose regimens have been projected. For increasing patient’s acceptance metformin is commenced at 500 mg every day as a single dose along with food. The dose is raised by 1000 mg after seven days and further it is again increased to 1500 mg. The dosage targeted is 1500–2550 mg/day which is given as 500 or 850 mg thrice daily. The effective response is perceived at the dose of 1000 mg once a day.
PCOS patients who do not respond to metformin at 1500 mg daily will positively respond to 2000 mg daily. Lactic acidosis is one of the adverse reaction noted in patients with renal failure, heart failure and sepsis.

Glueck et al showed that metformin has resulted in no main birth abnormalities and also no developmental defects in infants at the age of 3 and 6 months. One study compared the control group of women who was not treated with metformin and the treated group. They showed that the prevalence of diabetes during pregnancy in the metformin group was considerably less. In new PCOS, metformin compared with placebo shows improvement in ovulation rates. Metformin when compared with CC showed no advantage in ovulation, pregnancy, or live birth. In new PCOS, when metformin or clomiphene is given as single therapy they are more effective than the combination of metformin and clomiphene. In Clomiphene Citrate resistant patients, it has no advantage in ovulation and live birth amounts over placebo as monotherapy. The mixture of metformin and Clomiphene citrate enriched ovulation and pregnancy than CC alone. Pre-treatment with Metformin increases the effectiveness of this drug in PCOS patients.
Beyond its effect on glucose metabolism metformin also plays a role in reduction of plasminogen activator inhibitor (PAI)-1, von Willebrand factor (vWF), smooth muscle cell (SMC) contractility via agonist induced increase in intracellular (Ca^{2+}) or a secondary increase in nitric oxide\textsuperscript{75}.

Metformin also plays a role in inflammation by decreasing inflammatory markers in plasma including soluble intercellular adhesion molecule, vascular cell adhesion molecule-1, macrophage migration inhibitory factor and C-reactive protein (CRP) in case of Polycystic ovary syndrome. IL-8 plays a dominant part in recruitment of monocytes and adhesion to endothelial cells in atherosclerosis and IL-6 plays a role in acute phase response. Metformin hinders IL-1β stimulated IL-6 and IL-8 manifestation exhibiting the mechanism through which metformin therapy attenuates inflammation. In smooth muscle cells of human in atherosclerotic plaques there is stimulation of multipotent pro-inflammatory transcription regulator. Metformin acts by inhibiting NF-κB activation hence modulates inflammatory response to IL-1β\textsuperscript{76}.

EL Mekkawi SF, ELHosseiny AS, et al. showed that there was a decrease in interleukin-6 and interleukin-18 level after metformin
treatment for three months in polycystic ovary syndrome patients. This shows metformin decreases the inflammatory markers and have beneficial effect on inflammatory process of PCOS\textsuperscript{77}.

**Troglitazone** is also an insulin-sensitizing drug which improves the ovulation and the pregnancy. But, because of its liver toxicity, it has been taken away from the market. Alternative medication in the similargroup, rosiglitazone (8 mg/day) which shows to augment impulsive and also clomiphene-stimulated ovulation in PCOS women\textsuperscript{78}.

**Pioglitazone** also seems to be in effect. But its safety is also questioned. Foetal safety has not been recognised for both rosiglitazone and pioglitazone which comes under pregnancy category C of US FDA 2 guidelines.

Tang et al recently reviewed the insulin-sensitizing drugs like metformin, rosiglitazone, pioglitazone, and d-chiro-inositol for PCOS females, oligo/amenorrhea, and subfertility. They finalised that metformin is beneficial in the pregnancy and also ovulation. Though, there is no proof that metformin rises birth rates when this is given as monotherapy or combined with clomiphene. Thus, the usage of metformin in improving fertility consequences in women with PCOS seems to be limited\textsuperscript{79}.
Even small amounts of FSH administered into the circulation along with FSH injections or with pulsatile GnRH or clomiphene citrate, is adept of stimulating ovulation and pregnancy in a huge number of women with PCOS with anovulation. It has been in effect in ovulation induction for PCOS women and considered to be the main line treatment of those patients. **Clomiphene citrate** is a partial oestrogen agonist. Clomiphene citrate is strictly related to the oestrogen chlorotrianisene. Absorption of this compound through oral route is efficient. Its half-life is 5–7 days and is excreted through urine mainly. It has strong binding to protein and enterohepatic circulation and is circulated to adipose tissues.

The estrogenic agonistic actions are well seen in animals with gonadal deficiency. It blocks the oestrogenic actions. It causes an increase in the production of gonadotropins and oestrogens by blocking the negative feedback effect of estradiol on the gonadotropins. The importance of this drug is seen on its efficiency to induce ovulation in oligomenorrhea or amenorrhea women and disorder of ovulation. Clomiphene blocks the feedback inhibitory stimulus of oestrogens on the hypothalamus, leading to a rush of gonadotropins, which causes ovulation.

Generally, one ovulation is stimulated by a single course of drug, and the patient must be treated subsequently till the pregnancy is reached, as
normal ovulatory action does not usually occur. The drug has no role in ovarian or pituitary failure patients. When clomiphene is given in a dose of 100 mg/d for 5 days, an increase in plasma LH and FSH is seen after many days. In ovulatory patients, there is an early rise followed by another rise of gonadotropin levels just preceding to ovulation. Careful monitoring of ovarian response with serial ultrasound scans is essential and in those who respond sensitively to 50mg, a lower dose of 25 mg may be used. A duration of three to six ovulatory cycles is usually adequate to distinguish whether pregnancy will be attained using clomiphene citrate, before going on to a multifaceted treatment, as around 75% of the pregnancies attained with clomiphene citrate happen in the first three cycles of treatment\(^8^0\). If the patient is ovulating, it is not essential to raise the dose even if conception has not resumed, and conception is anticipated to happen at a rate determined by influences like the patient’s age. All women who are given with clomiphene should be cautiously observed with combined endocrine and ultra-sonography assessment of follicular growth and ovulation, for the reason of multiple pregnancy\(^8^1\).

Ovulation occur in about 80% but then end result of pregnancy is only approximately 35-40% of patients who are given clomiphene\(^8^2\).
Clomiphene citrate normalises LH and increase FSH secretion. This result in inducing follicle growth and ovulation. Various studies reported clomiphene to be the standard treatment for the patients of polycystic ovary syndrome for their induction of ovulation\textsuperscript{83, 84}. A meta-analysis showed use of clomiphene citrate in PCOS women compared to placebo has six times more potency to result in pregnancy\textsuperscript{83}. In practice, the usual regimen is an initial dose of 100 mg once a day from day 4 or 5 and this has no benefit by a daily dose of more than 150 mg which appears to considerably increase either the ovulation rate or follicular recruitment. This type of schedule will decrease the number of ‘superfluous’ cycles of therapy until those resistant to clomiphene are identified.

A prospective study on the follow-up of lean women with disorder of ovulation has reported more conception rates in patients responding to ovulation who are being treated with clomiphene. 50\% achieved ovulation after three cycles and 75\% after nine cycles of treatment\textsuperscript{83}.

Despite its efficacy some women do not ovulate and do not achieve pregnancy due to its adverse effects on endometrium like hyperandrogenemia, ovarian volume, menstrual irregularities and obesity. Also patients with increase basal LH have less chance to
respond to clomiphene\textsuperscript{85}. Kousta et al showed treatment with clomiphene citrate for 167 patients showed a rate of 67.3\% of conception over six months in patients who do not have any subfertility factors\textsuperscript{86}. These patients were continued with twelve cycles of therapy. They described a multiple pregnancy rate of 11\%, like to that referred in other series, and a miscarriage rate of 23.6\%, with those who had miscarriage because of high LH levels immediately after clomiphene administration.

Shoham and colleagues observed the hormonal profiles in forty one women cured with clomiphene citrate of whom twenty eight ovulated\textsuperscript{87}. In those who ovulated, 17 had normal patterns of hormone secretion and five of them conceived. 11 of them who showed abnormal patterns of hormonal profile, high levels of LH from day 9 until LH spur, along with premature luteinisation and high concentrations of estradiol throughout the cycle, did not conceive.

If pregnancy was not achieved even after 10-12 normal ovulatory cycles it is then apt to suggest the couple assisted conception. The addition of an ovulatory dose of human chorionic gonadotropin (hCG), 5000-10000 IU can be administered if the cause for non-ovulatory response is delayed LH surge or absent of it even in the occurrence of a well-
developed follicle. The repetitive administration of hCG at mid-cycle result in little enhancement of conception rates if given when follicle reaches a diameter of 18-24 mm.

Daily doses of dexamethasone, 0.5 mg at bedtime, as an aide to clomiphene citrate treatment, suppress the production of adrenal androgen and may stimulate receptiveness to clomiphene in former non-responders, commonly PCOS hyper androgenic women with increased levels of dehydroepiandrosterone sulphate (DHEAS) \(^88, 89\). But side effects are more with this method.

A systematic review by Misso M et al. shows that combination of Clomiphene Citrate and metformin are improved than placebo for increasing ovulation and pregnancy rates. But CC is superior to metformin for ovulation, pregnancy and live-birth rates, in PCOS patients with body mass index (BMI) \(>30\). A combined CC and metformin is more efficacious than metformin alone or CC alone, depending on the BMI and CC sensitivity of the patient\(^90\).

A Prospective trial was conducted to compare the ovulation and pregnancy rates in infertile CC alone and combined metformin and CC PCOS women for three cycles of escalating the dosage from 50 mg till 150 mg alone compared with CC and then another 150 mg for three
cycles. Another group were given the same amount of CC along with 1500mg of metformin. Up to six cycles ovulation or pregnancy was assessed by transvaginal sonography. Rate of ovulation and pregnancy in combination with metformin and CC was greater than CC alone. Moreover, the study showed metformin augmented the ovulatory rate in CC failures indicating increased responsiveness to CC.

Clomiphene citrate does not show any effect on inflammation in PCOS. Menstrual irregularities are the most common symptom that occur in polycystic ovary syndrome. Amenorrhoea or oligomenorrhea is the primary complaint. In normal menstrual cycle oestrogen will be produced during ovulation and progesterone will also be secreted. The shedding of endometrium occurs if pregnancy doesn’t occur. This is Secretory phase.

For this phase **progesterone** is necessary. In PCOS progesterone are not secreted and hence there will be no shedding of endometrium. If this is continued the lining of cells which are not shredded gets accumulated and can lead to endometrial hyperplasia. So regular shedding is necessary. This can occur by giving low dose of progestogens cyclically and this induces regular menstruation. It can be given every month during secretory phase alone in low dose. Progestin after entering into
the cell gets bind to progesterone receptors which are dispersed in the middle of the nucleus and the cytoplasm. The ligand-receptor complex gets fixed to a progesterone response element (PRE) to stimulate the transcription of gene. The specificity of reaction is available in the cell and also other cell-particular receptor co regulators and relating transcription factors. The progesterone-receptor complex forms a dimer formerly tying to DNA. Like the oestrogen receptor, it can form heterodimers and in addition to homo dimers between two isoforms, A and B. These isoforms are delivered by optional splicing of the same gene$^{92}$. 
Progesterone has little impact on protein metabolism. It empowers lipoprotein lipase action and appears to favour fat deposition. The consequences on carbohydrate metabolism are more distinct.

Progesterone increments basal insulin levels and the insulin reaction to glucose. There is normally no difference in carbohydrate tolerance.92

Receptor of the renal tubule, bringing about a reduction in Na+ reabsorption. This prompts to a high production of aldosterone by the adrenal cortex (eg, in pregnancy). Progesterone rises body temperature in humans. The mechanism of this process is not known, but a change in the hypothalamus on the temperature-regulating centers has been recommended. Progesterone also changes the function of the respiratory centers. The ventilatory response to CO₂ is raised by progesterone but synthetic progestins with an ethinyl group don’t have respiratory causes. This causes to an amountable decrease in arterial and alveolar PCO₂ in the pregnancy and in the luteal phase of the menstrual cycle. In the brain, progesterone have depressant and hypnotic effects too.

In breast, it is the reason for the development of the alveolobular secretory components. It also involves the preovulatory LH spur and leads to the maturation and secretory alterations in the endometrium which occur after ovulation.
Regular treatment with progestin can cause breast engorgement, rise in body temperature, headache, depression, weight gain, irregular menstrual cycle, breakthrough bleeding. Severe cases can lead to amenorrhoea.

The frequency of menstrual cycles is less significant than the occurrence of endometrial hyperplasia. Intermittent induction of menstruation utmost usually by progestogens or oral contraceptives, either cyclically or continuously, inhibits abnormal uterine proliferation. Use of **combined oral contraceptives (COCs)** is the usual treatment for the symptoms of polycystic ovary syndrome. The COCs help to prevent recurrent anovulatory bleeding by providing a progestin and by suppressing ovarian hormones and adrenal androgen production. They also, indirectly, increase sex hormone–binding globulin (SHBG). SHBG binds to androgens and reduces their circulating concentrations. Combined oral contraceptives decreases insulin sensitivity, impairs glucose tolerance, and alters lipid profiles in healthy women, but apparently it do not affect the risk of diabetes mellitus or cardiovascular disease\textsuperscript{92}.

Combined oral contraceptive pills contain low dose oestrogens and progestin so that side effects are minimised. If the patient contain
hirsutism and acne more estrogenic preparation can be preferred. There are monophasic, biphasic and triphasic combination pills\textsuperscript{92}.

Monophasic pills contain no phasic increase or decrease in the oestrogen/progestin content during 21 days of pill administration. The first pill is taken on the fifth day after the start of the menses. Thereafter, one pill is to be taken each day consecutively for 21 days. Then, next seven days are “pill free period” and the next course starts again after the fifth day of menses. On repeating this schedule, the cycle becomes anovulatory (due to estrogenic effect) with a regular cyclic menstrual bleeding on withdrawal of the pill (due to progestogenic effect). Biphasic and triphasic pills mimic the hormonal changes during the menstrual cycle as physiologically as possible. Biphasic pills have a fixed dose of oestrogen for twenty one days but with increasing doses of progesterone during two successive phases i.e. from the day one to ten and eleven to twenty one. Next seven days are pill free days. Triphasic pills provide a bit higher dose of oestrogen at near mid cycle but increasing doses of progesterone for three successive phases i.e. for days one to six, seven to eleven and twelve to twenty one days.

The combination pills supress the ovulation by inhibiting the release of FSH and LH [mainly estrogenic effect]. High doses of oestrogen also
inhibit LH surge. The role of progesterone is to ensure withdrawal bleeding after stopping the medication. Normal ovulatory cycle is regained within one to three months of stopping the COC \(^9^2\).

COCs can also be combined with **Cyproterone acetate (CPA)** which is an anti-androgen. This not only regulates menstrual cycle but also effective in reducing the symptoms of hyperandrogenism such as hirsutism and acne. This in turn prevents endometrial hyperplasia. Cyproterone acetate (CPA) act chiefly by meddling with target tissue binding of androgens to their intracellular receptor. They are used as monotherapy or as combined therapy with COCs for masculinizing signs that are not reactive to a single drug. Anti-androgens will improve sensitivity to insulin slightly in PCOS women \(^9^3\). They competitively blocks receptors of androgen intracellularly, but it has less activity than spironolactone. The fall in LH-dependent androgen synthesis is ascribed to the progestational activity of CPA and **ethinyl estradiol (EE)** that is given with it to deliver contraception and also blocks LH. Upto 50-90% patients a clinical effect in hirsutism if CPA has been taken regularly for 2-3 months. 35\(\mu\)g of EE and 2 mg of CPA is mostly combined. As PCOS is a progressive syndrome, at least up to the age of forty, it is necessary to treat the patient with EE and CPA. This combination can
reduce androgen levels distinctly and also their adverse reactions. It will make the disease ‘on hold’. So that the fertility rate increases even when the drug is discontinued. Other contraceptive drugs given periodically will regularize the periods and should be substituted after six months with the higher dose EE/CPA combination—since there is a chance of high possibility of thromboembolism. Cyclical progesterone therapy (using the less androgenic progestin such as medroxyprogesterone acetate and dehydrogesterone) may also be used to induce regular menstruation. A progesterone releasing IUCD may also be used to provide endometrial protection.

Falsetti L and Passinetti E studied the effects of combination of 0.035 mg of ethinylestradiol and 2 mg of cyproterone acetate on lipid metabolism in women with polycystic ovary syndrome (PCOS). For this, 72 PCOS women were given with thirty six repetitive cycles of EE and CPA. Profiles of hormones, lipid and sugar were read before and after 12 and 36 cycles of treatment. The treatment repressed gonadotropin and androgen levels and improved the concentrations of sex hormone binding globulin. After 12 and 36 cycles a substantial raise in triglycerides, high density lipoprotein (HDL) cholesterol system, and apoprotein B levels were perceived. Insulin and plasma glucose levels
had no change. During management triglycerides, total cholesterol and apoprotein B levels were more than the control group. There were no change in concentrations of LDL-C and HDL-C in PCOS and in the control group. Lipid and lipoprotein alterations seen after treatment can be because of oestrogen dominance of the treatment\textsuperscript{94}.

Maturation of oocytes is the final process of an immature female egg cells before fertilisation either in normal or stimulated ovarian cycles. Gonadotropins are the next line treatment for fertility in anovulatory women with PCOS\textsuperscript{95}. \textbf{Follicle Stimulating Hormone (FSH)} stimulates growth of follicles, ova development and production of oestrogens. \textbf{Luteinizing Hormone (LH)} stimulates preovulatory expansion of the ripe Graafian follicle and induces ovulation and then leutinization of the ruptured follicle and corpus luteum is sustained till the next menstrual cycle. Progesterone secretion occurs only under the influence of LH. GnRH action gonadotropins occurs via a G-protein coupled receptor which exerts its action by stimulating intracellular Ca\textsuperscript{2+} through hydrolysis of phophotidyl inositol phosphate\textsuperscript{96}. The production of gonadotropins raises at puberty and is more in women compared to men. In the follicular phase, moderate levels of FSH and low levels of LH will be present. There is a mid-cycle spur of both, but more of LH, just
before ovulation, followed by subsequent decrease in the luteal phase. Inadequate gonadotropins secretion results in amenorrhoea and sterility in women. However excess production of gonadotropins in women causes polycystic ovaries. The gonadotropins causes induction of ovulation, maintenance and incite ideal growth of follicle through a regulated injection of FSH, and attain a follicular size which is able to get fertilized. Gn doesn’t put forth an antiestrogenic action peripherally. The major disadvantage of Gn is the formation of many follicles so that increases the ovarian hyper stimulation syndrome (OHSS) and multiple pregnancies. FSH injection requires skill and rigorous monitoring and also very expensive and needs more time. Various therapy designs are employed like step-up, low-dose step-up, and step-down regimens. The ASRM vouch for low-dose gonadotropin protocols. The step-up regimen starts with dosage from 37.5–50 IU/day which is minimum and this is being increased regarding the deficiency of follicular response. The response are monitored using ultrasound and then treatment is altered 1 week later if there is no follicular development with an increase of 50% every time as needed.

HCG is useful as a substitute in the LH spur which causes maturation of the oocyte, follicular rupture and corpus luteal production. The step-
down course commences with the extreme suggested dose, which is then decreased as a follicle response is attained. The dose is reduced 50% every time when the therapy is altered. Current reports described higher protection for patients using the step-up regimen\textsuperscript{100}.

In 2006, the ASRM implied severe control when blood estradiol concentrations crossed 2500 pictogram/mL at the time of induction\textsuperscript{99}. Recent suggestions advise withholding hCG injection in the availability of greater than two follicles >16 millimetre or greater than one follicle >16 millimetre and two extra follicles >14 millimetre, or if serum estradiol concentrations are within 1000 and 2500 pictogram/mL, especially in female less than 38 years old who had no other infertility causes. So low-dose regimens ended in a monofollicular ovulation rate of $\sim70\%$, a pregnancy rate of 20\% each cycle, and multiple live birth of 5.7\% even then the incidence of multiple pregnancies was around <6\% and OHSS was around<1\%\textsuperscript{101}. A maximum of six cycles with gonadotropins is suggested since no action even for six cycles suggest their presence of resistance.

It is mainly attempted to stimulate ovulation along clomiphene citrate when failed or during anovulation which can be because of polycystic ovaries.
The usually used dosage of hCG in IVF is 10,000 International Units but studies shows 5,000 International Units is sufficient to yield similar results\textsuperscript{102}. These hormones act on women for their fertility and ovulation and not on inflammation.

In 1850 Johannes Joseph Scherer\textsuperscript{103} was the first man to isolate hexahydroxycyclohexane from muscle and named it Inositol as it belongs to the sugar family\textsuperscript{104}. The hexahydroxycyclohexane structure has 9 different stereo isomers. Among them, \textbf{Myo-inositol} is the most distributed isomer in the body. It has both metabolic and functional importance. Myo-inositol is thought to be a prebiotic molecule\textsuperscript{105} and inositol and inositol-derivatives has various roles in biological systems. Later inositol was found to be the main component of phytates which is the salts of inositol hexaphosphoric acid. In 1914, Anderson\textsuperscript{106} showed the molecular structure of myo-inositol- 1, 2, 3, 4, 5, 6-hexakis dihydrogen phosphate which is said also as phytic acid and confirmed by various modern analytical methods\textsuperscript{107}.

Inositol is found in foods like fruits and beans\textsuperscript{108}. Myo-inositol is incorporated into the cell wall as phosphatidyl myo-inositol, the pioneer of inositol triphosphate which acts as a second messenger regulating the mechanism of hormones such as FSH, TSH and insulin. Addition to it,
inositol is an important component of the structural lipids. After Scherer, many researchers started to study the role of inositol in different organs and tissues specifically its role in cell shape and oocyte fertility. In 1964, Eisenberg et al., and Eisenberg and Bolden found that testes are rich of free inositol; later, Voglmayr and Amann, Lewin and Beer, and Ghafoorunissa reported that the prostate, the epididymis and seminal vesicles contain a large amount of myo-inositol. The seminal fluid is one of the richest sources of inositol. The concentration of inositol in seminal fluid is almost three times higher than that found in plasma.

These primary findings gave the first indirect proof relating inositol based molecules to germ-cell (spermatozoa and oocytes) physiology. In 1974, Larner reported the presence of various intracellular chemical mediators of insulin, and hypothesized that, after the binding of insulin to its receptor; different intracellular pathways could be triggered according to the specific mediator involved. In 1988 Larner et al. was drawn to a conclusion that the two inositol stereo isomers, Myo-inositol and D-chiro-inositol, are present. They are the chemical mediators of insulin. These both act through different mechanisms but
have same forms, changing only in the stereochemistry of having one hydroxyl group\textsuperscript{119}.

Natural sources for these inositols are endogenous biosynthesis and dietary intake. Myo-inositol is produced from glucose-6-phosphate in two steps. First, glucose-6-phosphate isomerizes to myo-inositol-1-phosphate, which is dephosphorylated by an inositol monophosphatase enzyme giving free myo-inositol\textsuperscript{120}.

**Picture 5: Formation of Myo-inositol**

\begin{center}
\includegraphics[width=0.5\textwidth]{formation_of_myoinositol.png}
\end{center}
In vivo, **D-chiro-inositol** is synthesized by an epimerase that converts myo-inositol into D-chiro-inositol.
Larner first studied a decreased D-chiro-inositol content in urine as well as tissues of human subjects and animals with type 2 diabetes\textsuperscript{119, 121}. He postulated that the myo-inositol when converted to D-chiro-inositol requires stimulation of insulin\textsuperscript{122}. This conversion is decreased in the insulin sensitive tissues like fat, liver, muscles of rats\textsuperscript{123}.

PCOS pathogenesis is linked to insulin resistance and/or compensatory hyperinsulinemia irrespective of their BMI whether they are lean or obese. The impairment in the insulin signaling in PCOS could be due to
a defect in the inositol phosphoglycans (IPGs) second messenger pathway\textsuperscript{119} opened a new angle in the clinical management of PCOS. IPGs have a role in stimulating enzymes that regulate glucose metabolism\textsuperscript{124, 125}. In PCOS women, a defect in tissue availability or altered metabolism of inositol or IPGs mediators may contribute to insulin resistance\textsuperscript{126}. In 1999, Nestler JE et al. published Ovulatory and metabolic actions of D-chiro-inositol in the polycystic ovary syndrome assessed steroids level in serum and oral glucose tolerance tests before and after the oral administration of 1200 mg of D-chiro-inositol or placebo for six to eight weeks in 44 obese PCOS patients once daily. The results proved that PCOS patients who received D-chiro-inositol has improved insulin sensitivity and serum free testosterone levels were reduced than the placebo group. In addition to it, diastolic and systolic blood pressure, and plasma triglyceride levels were decreased in patients treated with D-chiro-inositol. Ovulation occurred in 19 out of 22 women (86\%) who received D-chiro-inositol, as compared to 6 out of 22 (27\%) in the placebo group\textsuperscript{127}.

In 2002, Nestler and Allan reported a clinical study in which they saw whether administration of D-chiro-inositol would affect the levels of circulating insulin and androgens, and the occurrence of ovulation in
lean PCOS patients. These results confirmed earlier findings which showed in lean PCOS women also, D-chiro-inositol reduces serum insulin and androgen and also improves PCOS-associated metabolic abnormalities (increased blood pressure and hypertriglyceridemia).\(^{128}\)

Chiu et al in 1992 published a study which showed an increased concentration of inositol in patients with successful IVF pregnancies. This indicates the involvement of inositol in the early phase of in vitro IVF and also for the maintenance of normal development of embryo. They also reported that patients who had successful IVF pregnancies and also those who had high levels of myo-inositol showed the development of the embryos with higher number of somities.\(^{129}\) Same group of authors\(^ {130}\) after ten years conducted a study to examine whether the content of myo-inositol in human follicular fluid was associated with good oocyte quality. For this fifty three patients who had been treated with IVF were recruited and their follicular fluid and serum were collected. Then they have been divided into two groups with group A consisting follicular fluid with matured and fertilized oocytes and group B has immature follicles and unfertilized oocytes. There was a statistical significance of correlation between the levels of myo-inositol in the follicular fluid and the quality of oocytes. This showed greater follicular
concentration of myo-inositol is associated with greater follicular maturity. So concentration of myo-inositol is a quality marker for evaluation for oocytes.

Unfer et al. conducted a study on myo-inositol administration in PCOS patients. Twenty five patients participated in this study and they were given myo-inositol and folic acid twice daily. Their ovulatory activity was seen through sonography and hormonal profile, number of spontaneous menstrual cycles and pregnancies were noted. The results obtained showed the efficacy of myo-inositol in simultaneous ovarian activity and fertility in PCOS patients\textsuperscript{131}.

Following this study many authors\textsuperscript{132, 133} reported daily intake of myo-inositol improves hormonal profile, restores ovulation, and induces regular menses in PCOS patients who are both lean and obese.

Rizzo et al. reported the efficacy of myo-inositol and folic acid in fertility improvement in PCOS patients compared to Myo-inositol plus folic acid and melatonin. Unfer et al. again showed there was no difference in myo-inositol and D-chiro-inositol treated groups on the number of oocytes but mature oocyte number was increased and the immature oocytes got decreased in myo-inositol group\textsuperscript{134}. 

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In patients with PCOS there was a myo-inositol depletion which resulted in poor oocytes\textsuperscript{135}. This myo-inositol depletion resulted in impairment of FSH signaling in PCOS patients\textsuperscript{136}. Various studies reported shows the need of rFSH got decreased during IVF cycles when myo-inositol supplemented three months before the stimulation of ovary\textsuperscript{134, 135, 137}.

Isabella and Raffone\textsuperscript{140} showed increase in D-chiro-inositol will produce ovary toxicity which is characterized by poor quality of oocyte and reduced response of ovary to FSH. Another study by Galetta M et al. showed healthy volunteers had high myo-inositol concentration and low D-chiro-inositol concentration. But PCOS patients had opposite ratio. This is called “DCI paradox”\textsuperscript{141}.

Nowadays combined treatment of D-chiro-inositol and myo-inositol are also being given. Minnozi et al. showed the combined therapy of myo-inositol and D-chiro-inositol was able to improve the metabolic consequences of PCOS women, thus decreasing the cardiovascular risk factors. In this study twenty obese PCOS patients were taken and their lipid profile such as HDL, LDL, Triglycerides were assessed before and after six months of treatment with combination therapy. Changes in body mass index, waist-hip ratio, body fat percentage, blood pressure
and HOMA-IR were evaluated. There was a strong increase in HDL levels and decrease in LDL and Triglycerides levels. Reduction in HOMA-IR was also seen concluding that the combination therapy improves the metabolic profile\(^{142}\).

**N-acetyl cysteine (NAC)** acts by inhibiting the stress of oxidation and preventing the increased glucose levels which stimulates the resistance of insulin. A study by Fulghesu et al.\(^{143}\) illustrated the patients with PCOS who had increased insulin levels when treated with N-acetyl cysteine, treatment with NAC resulted in progress of insulin sensitivity in addition to marked decrease in concentrations of testosterone and lipids. Kilic-Okman and Kucuk\(^{144}\) has showed NAC decreases serum testosterone and insulin levels and improved homocysteine in addition to lipids in PCOS patients. Study by Rizk et al.\(^{145}\) reported the combination of CC and NAC rises the rates of both ovulation and pregnancy in infertile CC-resistant patients infertile. N-acetyl cysteine plus clomiphene citrate when compared with clomiphene citrate alone showed improved follicular growth. Thus emphasizing that N-acetyl cysteine is effective in inducing and maturing the oocytes. A study by Saghar Salehpour et al.\(^{146}\) reported that N-acetyl cysteine induces enhancement in profile of lipids, hormones, rate of ovulation, and as a
result the health outcomes of PCOS women. This is via the block stress of oxidation in addition to increasing the sensitivity of insulin in the tissues peripherally. The adverse drug events were very less due to NAC and hence it is taken into consideration for treatment of PCOS women.

As described earlier it is not only the anovulation but also decrease in the quality of oocyte and embryo were the reason for infertility in PCOS women. Poor oocyte quality is due to ROS-induced oxidative stress. Raise in lipid peroxidation products in serum as well as in follicular fluids were studied by various authors\textsuperscript{147,148}. Sun C.L.et al showed apoptotic granulosa cells is more in PCOS females\textsuperscript{149}. 
Increased oxidative stress

Lipid peroxidation
Protein oxidation
DNA damage

Damage to granulosa cells
and oocyte

Melatonin
The major enzymatic metabolite of melatonin 6-sulfatoxymelatonin levels in urine is more in PCOS women than the female without PCOS\textsuperscript{150}. LH production, and LH reaction to GnRH were enhanced due to elevated melatonin levels\textsuperscript{150}. This high levels of melatonin may be due to feedback action to the lack of melatonin in the ovary. With this current understanding of actions melatonin may be a potential treatment for PCOS in the future.

\textbf{Statins} are the blocks of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase. It is the rate limiting process in the synthesis of cholesterol pathway. They strongly inhibit the secretion of cholesterol which is a substrate for testosterone as well as improves hyperandrogenemia. Apart from this they limit the mechanisms of insulin and IGF-1 on the ovary. This is done with the help of reducing N-linked glycosylation so that there will be maturation of insulin and Type 1 IGF-1 receptors. Not only this they also reduce isoprenylation of small GTPases such as Ras and Rac which are the mediators of insulin signaling pathway\textsuperscript{151}.

Statins blocks the mevalonate pathway which lead to abolition of the actions of increase of insulin levels on the proliferation and
steroidogenesis of thecal cells. They also inhibit the oxidative stress which causes increase in proliferation of cells, steroidogenesis and insulin resistance. So these two roles result statins to have a better effect in the PCOS and its effect on the cardiovascular mortality and morbidity.

**Laparoscopic ovarian drilling** is the surgical method in women with clomiphene-resistant PCOS and who persistently hyper secrete LH. After the surgery actions of ovary is restored and concentrations of LH and testosterone level fall\(^{152}\). Hence surgery helps to bring down the levels of LH and androgen even in patients with elevated LH concentrations. So laparoscopic ovarian drilling is more effective in patients with menstrual irregularities and increases the fertility rate in women who come to infertility clinics\(^ {153}\).

Even though these many drugs and modalities of treatment were available for PCOS, only few drugs targeted in reduction of inflammation. There were various studies, as we quoted earlier, demonstrating the effect of inflammation on PCOS. These studies were all done in other parts of the world. There were very less number of studies done in India. So we proposed to study the status of inflammation on PCOS in Indian population in particular South Indian
population. There were also as we said earlier studies on drugs targeting on inflammation in PCOS. These studies mainly spoke about metformin and their anti-inflammatory properties. There were no studies demonstrating the anti-inflammatory effect of other drugs used to treat PCOS in practise.
MATERIALS AND METHODS

This study was a **prospective cross sectional study**. The study population was from all the patients who attended the outpatient Department of Obstetrics and Gynecology in PSGIMSR Hospital, Coimbatore. We included all the PCOS patients according to Rotterdam consensus criteria with age group 18-45 years and excluded those patients presenting with a history of inflammatory diseases or intake of inflammatory drugs and also pregnant women. Healthy Volunteers of age group 18-45 years women with normal reproductive cycle were included and those who present with history of inflammatory drugs or diseases are excluded. Further those who have hypertension or diabetes are also excluded from the healthy volunteers.

The overview of the study design is given in the figure below. The patients were divided into three groups.

**Group 1:** Healthy volunteers

**Group 2:** Newly diagnosed PCOS

**Group 3:** Already on treatment for PCOS

Sample size estimated according to previous literature search.30 participants in each group was taken.
Healthy female volunteers

- 30 volunteers
  - Blood tests
    - hsCRP
    - IL-6
    - IL-18

Newly diagnosed PCOS

- 30 patients
  - Blood tests
    - hsCRP
    - IL-6
    - IL-18

Already on treatment for PCOS

- 30 patients
  - Blood tests
    - hsCRP
    - IL-6
    - IL-18
The study design was submitted to the Institute Human Ethics Committee (IHEC) stating its purpose preceding the study. The details and the usefulness of the study design were described to every participant individually and clarified their doubts before getting the informed consent forms. The informed consents forms provided to the participants were either in English or in Tamil. The participants, who gave written informed consent, came under the inclusion criteria were enrolled for the study. Healthy volunteers were also given the informed consent. The copy of consent forms were attached in the annexure. The patients who declined to give informed consent were also excluded from the study.

PCOS patients were selected according to Rotterdam Consensus Criteria 2003 which is given below.

- *Polycystic ovaries*(12 or more follicles in each ovary, each follicle measuring 2-9 mm in diameter and/or ovarian volume > 10 ml, one polycystic ovary is sufficient for diagnosis)

- *Oligo-/anovulation; clinical diagnosed as Oligo-/amenorrhea (menstrual cycles longer than 35 days or less than 10 menstruation/year)*
• Hyperandrogenism (clinical or biochemical)

Basic demographic data like name, age and anthropometric measurements of Height, weight, body mass index were recorded. History of the patient, any co morbidities was noted. If the patient is on already treated group then their treatment history were noted.

Our study was to observe if there were abnormalities in High sensitivity C - reactive protein, Interleukin-6 and Interleukin-18 in PCOS patients and to compare those with the healthy volunteers.

Sample for High sensitivity C - reactive protein (hs-CRP) was collected in red topped vacutainer which is a tube without any anticoagulant. The blood samples for Interleukin -6 (IL-6) and Interleukin -18 (IL-18) were collected in yellow topped vacutainer which had acid citrate dextrose as anticoagulant.

The high sensitivity CRP was tested in the Clinical Biochemistry lab using the Cobas Integra C - reactive protein (Latex). This machine works by the methodology of “Particle enhanced turbidimetric assay”. When Human CRP is applied over the monoclonal anti-CRP antibodies, it agglutinates due to exposure of latex particles. The precipitate is determined turbidimetrically at 552nm.

Sample collected
was centrifuged and the serum was used for analyses. The analyzer automatically calculates the analyte concentration of each sample. The value <0.05mg/dl was considered to be normal.

The Interleukin-6 was estimated in the Pharmacology laboratory using Krishgen Biosystems, imported from California, USA. The IL-6 enzyme immunoassays are programmed for measuring the quantity of human interleukin-6(IL-6). Samples and other requirements used for quantification are coated in microtiter plate which are previously coated with first monoclonal Ab, anti IL-6 in the presence of second Ab linked to streptavidin Horseradish peroxidase (streptavidin-HRP). Later when the incubation period is over these wells are washed and with the help of a colored substrate the enzymatic activity is detected. This color is proportional to the Interleukin-6 concentration in the serum sample.

Serum was obtained by allowing the blood to clot for 30 minutes and by centrifuging it for 10 minutes at 1000 x g. Then they were stored. Then the serum was separated carefully from clot in a separate plastic tubes and stored at <-20 degree C.

The kit contains a plate with ready to use 96 wells. The reagents were prepared for assaying IL-6. 5 ml of 20X wash buffer was added to 95 ml and 1 ml of 5X Assay diluents to 4 ml of distilled water.
**microliters of Detection Ab** to 9975 microliters of 1X Assay Diluents to get a final volume to 10 ml and **50 microliters of streptavidin-HRP** to 9950 microliters of 1X Assay Diluents to get final volume to 10 ml were added. **Recombinant protein** was diluted by adding 20 microliters of std. solution in 10 ml of 1X Assay Diluents and top standard solution 2000pg/ml was prepared. Six two-fold serial dilutions were prepared from the top standard solution 2000picogram/ml such that concentrations are 1000picogram/ml, 500picogram/ml, 250picogram/ml, 125picogram/ml, 62.5picogram/ml, 31.3picogram/ml and 1X assay diluents serve as zero standard.

100µl/well of standards and samples were added to the plate. After sealing it was incubated at 37 degree C for 1 hour and 30 minutes. The wells were washed at least for 4 times using the wash buffer 1X and the
plate was turned upside-down and tapped firmly onto a clean absorbent paper. 100 µl of diluted Detection Ab was added to all wells and the plate was kept for incubation at 37 degree c for 90 minutes. Again the wells were washed using wash buffer 1X and 100 microliters of diluted streptavidin-HRP was added to each well after which the plate was sealed and incubated at 37 degree c for 30 mins. Wells were washed for four times following which it was soaked in wash solution for 30 to 60 seconds in between every wash to minimize the background error. Finally, freshly prepared TMB substrate 100 microliters was added to each well and was kept in the dark for 15 to 30 minutes. The wells showing positive reaction turned blue in color after which the reaction was stopped by adding 100 microliters of stop solution.

The plate was then inserted into an ELISA reader and the absorbance read at 450 nm within 30 minutes of adding stop solution. ELISA reader is a Bio-Rad system which is attached to a computer which has curve fitting software. The unknown concentrations of interleukin-6 were then identified by plotting the graph.

The Interleukin-18 was assessed in the Pharmacology laboratory using Krishgen Biosystems, imported from California, USA. This ELISA kit is based on the principle of double-antibody sandwich ELISA technique.
to detect Interleukin 18 (IL-18) in body fluids. The kit uses a double-antibody sandwich enzyme-linked immuno-sorbent assay (ELISA) to determine the amount of Human Interleukin 18 (IL-18) in a biological sample. Samples are added to micro-plate wells pre-coated with Human Interleukin 18 (IL-18) antibody. After incubation and washing, Human Interleukin 18 (IL-18) detection antibodies labeled with biotin is added. After incubation and washing Streptavidin-HRP is added to form immune complex, followed by incubation and washing to remove the uncomplexed enzymes. Then chromogenic TMB substrate solution is added, the color of the liquid changes to blue. Stop HRP enzyme reaction by addition of acid stop solution, when color changes to yellow. The intensity of color developed is proportional to the concentration of the Human Interleukin 18 (IL-18) present in the sample and read at 450nm using a plate reader.

Serum was obtained by allowing the blood to clot for 30 minutes and by centrifuging it for 10 minutes at 1000 x g. Then they were stored. Then the serum was separated carefully from clot in a separate plastic tubes and stored at <-20 degree C.

The kit contains a plate with ready to use 96 wells. The reagents were prepared for assaying IL-6. 5 ml of **20X wash buffer** was added to 95
ml and 1 ml of 5X Assay diluents to 4 ml of distilled water. 25 microliters of Detection Ab to 4975 microliters of 1X Assay Diluents to get a final volume to 10 ml and 50 microliters of streptavidin-HRP to 9950 microliters of 1X Assay Diluents to get final volume to 10 ml were added. Recombinant protein was diluted by adding 20 microliters of std. solution in 10 ml of 1X Assay Diluents and top standard solution 2000 pg/ml was prepared.

Six two-fold serial dilutions were prepared from the top standard solution 2000 picogram/ml such that concentrations are 1000 picogram/ml, 500 picogram/ml, 250 picogram/ml, 125 picogram/ml, 62.5 picogram/ml, 31.3 picogram/ml and 1X assay diluents serve as zero standard.
100µl/well of standards and samples were added to the plate. After sealing it was incubated at room temperature for 90 minutes. The wells were washed at least for 4 times using the wash buffer 1X and the plate was turned upside-down and tapped firmly onto a clean absorbent paper. 100µl of diluted Detection Ab was added to all wells and the plate was kept for incubation at room temperature for 90 minutes. Again the wells were washed using wash buffer 1X and 50 microliters of diluted streptavidin-HRP was added to each well after which the plate was sealed and incubated at room temperature for 30 mins. Wells were washed for four times following which it was soaked in wash solution for 30 to 60 seconds in between every wash to minimize the background error. Finally, freshly prepared TMB substrate 100 microliters was added to each well and was kept in the dark for 15 minutes. The wells showing positive reaction turned blue in color after which the reaction was stopped by adding 100 microliters of stop solution.

The plate was then inserted into an ELISA reader and the absorbance read at 450 nm within 30 minutes of adding stop solution. ELISA reader is a Bio-Rad system which is attached to a computer which has curve fitting software. The unknown concentrations of interleukin-6 were then identified by plotting the graph.
**Statistical Analysis:**

Statistical analysis were performed by using **SPSS software version 19**. The results were analyzed using **one way ANOVA** and post hoc analysis was done using **LSD**.
RESULTS

We included the patients who attended Obstetrics and Gynecology department and diagnosed to have PCOS. Their history when asked was more likely of amenorrhea or infertility. Of these, regarding infertility patients when history was asked it was found those patients have history of oligo/amenorrhea. Other patients were excluded.

The statistical analysis done was One-way ANOVA and post hoc LSD using SPSS software version 19.
Figure 1 shows the age distribution of the study population

There were no patients above 40 years of age. 73.3% of newly diagnosed patients and 66.67% of already diagnosed patients are in the 3rd decade.
43.33% of newly diagnosed and 50% of already diagnosed and 53.33% of normal healthy volunteers were normal. 36.67% of newly diagnosed and 33.33% of already diagnosed and 30% of normal healthy volunteers were overweight. 13.33% of normal and already diagnosed patients were obese and only 3.33% of newly diagnosed patients were obese.
Figure 3 represents the history of the patients

The patients attended the outpatient department either for menstrual abnormality or infertility treatment.

66.67% of newly diagnosed and 60% of already diagnosed patients have attended outpatient department for menstrual abnormality either for oligomenorrhea or amenorrhea. Rest of the patients came for infertility treatment.
Figure 4 represents the marital status of the study population. 60% of the newly diagnosed patients and 80% of the already diagnosed patients were unmarried. 40% of the newly diagnosed patients and 20% of the already diagnosed patients were married.
Figure 5 represents the newly diagnosed study population with thyroid disease.

6.67% of the newly diagnosed population have thyroid.
Figure 6 represents the already diagnosed study population with thyroid.

6.67% of the already diagnosed population have thyroid.
Figure 7 represents the newly diagnosed study population with prolactin levels.

50% of the newly diagnosed study population had normal prolactin levels.
Figure 8 represents the already diagnosed PCOS study population with Prolactin levels.

36% of the study population had normal prolactin levels.
Figure 9 represents the therapies in the already diagnosed group.
Table 1 represents the percentage of drug treatment in the same group

<table>
<thead>
<tr>
<th>Drug</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metformin Combination</td>
<td>70</td>
</tr>
<tr>
<td>Myoinositol Combination</td>
<td>33.33</td>
</tr>
<tr>
<td>Hormones</td>
<td>23.33</td>
</tr>
<tr>
<td>Clomiphene citrate</td>
<td>20</td>
</tr>
<tr>
<td>Human Chorionic Gonadotropin injection</td>
<td>13.33</td>
</tr>
<tr>
<td>Laprosopic Ovarian Drilling</td>
<td>3.3</td>
</tr>
</tbody>
</table>

70% of the study population in already diagnosed group were on Metformin combination and 33.33% of the study population were in Myoinositol Combination.
Figure 10 represents the combination of drugs.

Table 2 represents the combinations of drug.

<table>
<thead>
<tr>
<th>COMBINATION TREATMENT</th>
<th>Met</th>
<th>Met + Myo</th>
<th>Myo + Hcg</th>
<th>Myo + Hcg + Clomi</th>
<th>Myo + Clomi + Met</th>
<th>Myo + Hcg + Clomi</th>
<th>Met + Hcg</th>
<th>Met + Clomi</th>
<th>Myo + Clomi</th>
<th>Met + Myo</th>
<th>Myo + Hcg + Surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Met</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Met + Myo</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Myo + Hcg</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myo + Hcg + Clomi</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myo + Clomi + Met</td>
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</tr>
<tr>
<td>Myo + Hcg + Clomi</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Met + Hcg</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Met + Clomi</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myo + Clomi</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Met + Myo</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myo + Hcg + Surgery</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Met – Metformin
Myo – Myo-inositol
Clomi – Clomiphene citrate
Hcg – Human Chorionic Gonadotropin
<table>
<thead>
<tr>
<th>Drugs</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Only Metformin</td>
<td>46.67</td>
</tr>
<tr>
<td>Metformin + Myoinositol</td>
<td>13.33</td>
</tr>
<tr>
<td>Myoinositol + Clomiphene citrate</td>
<td>10</td>
</tr>
<tr>
<td>Metformin+ Clomiphene citrate</td>
<td>6.67</td>
</tr>
<tr>
<td>Myoinositol + HCG + Clomiphene citrate</td>
<td>6.67</td>
</tr>
<tr>
<td>Myoinositol + Metformin + Clomiphene citrate</td>
<td>6.67</td>
</tr>
<tr>
<td>Myoinositol + HCG</td>
<td>3.33</td>
</tr>
<tr>
<td>Metformin + HCG</td>
<td>3.33</td>
</tr>
<tr>
<td>Myoinositol + HCG + LOD</td>
<td>3.33</td>
</tr>
</tbody>
</table>

46.67% of patients were prescribed with Metformin alone and metformin along with myoinositol were prescribed for 13.33% of patients. Myoinositol with Clomiphene citrate were given for 10% of patients and Metformin and Clomiphene citrate were given for 6.67% of patients.
Figure 11 represents the duration of treatment

26 patients (86.67%) were on treatment for less than 1 year.
Table 3 and Table 4 represents the comparison of hs-CRP for the three groups.

Table 3: One way ANOVA:

<table>
<thead>
<tr>
<th></th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between groups</td>
<td>0.025</td>
<td>0.975</td>
</tr>
<tr>
<td>Within Groups</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4: Post Hoc (LSD) analysis:

<table>
<thead>
<tr>
<th>Between Groups Comparison</th>
<th>Mean Difference</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>I and II</td>
<td>0.00667</td>
<td>0.963</td>
</tr>
<tr>
<td>I and III</td>
<td>0.02433</td>
<td>0.867</td>
</tr>
<tr>
<td>II and III</td>
<td>0.03100</td>
<td>0.831</td>
</tr>
</tbody>
</table>

There was no difference between the newly diagnosed and already diagnosed study population and there was no statistically significant decrease in the hs-CRP level in between the groups.
Figure 12 shows the hs-CRP levels for the three groups.
Table 4 and Table 5 represents the IL-6 level. There was no difference between the newly diagnosed and already diagnosed study population and there was no statistically significant decrease in the IL-6 level.

**Table 4: One-way ANOVA:**

<table>
<thead>
<tr>
<th></th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between groups</td>
<td>0.293</td>
<td>0.746</td>
</tr>
<tr>
<td>Within groups</td>
<td>0.746</td>
<td></td>
</tr>
</tbody>
</table>

**Table 5: Post hoc (LSD) analysis:**

<table>
<thead>
<tr>
<th>Between Groups Comparison</th>
<th>Mean Difference</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>I and II</td>
<td>56.23703</td>
<td>0.746</td>
</tr>
<tr>
<td>I and III</td>
<td>57.06563</td>
<td>0.506</td>
</tr>
<tr>
<td>II and III</td>
<td>0.82860</td>
<td>0.992</td>
</tr>
</tbody>
</table>
Figure 13 represents the IL-6 levels between three groups.
Table 6 and Table 7 represents the IL-18 levels. We were not able to establish a impact between the two population.

**Table 6: One way ANOVA:**

<table>
<thead>
<tr>
<th></th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between groups</td>
<td>1.954</td>
<td>0.148</td>
</tr>
<tr>
<td>Within Groups</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 7: Post hoc (LSD) analysis:**

<table>
<thead>
<tr>
<th>Between Groups Comparison</th>
<th>Mean Difference</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>I and II</td>
<td>133.417</td>
<td>0.114</td>
</tr>
<tr>
<td>I and III</td>
<td>150.75323</td>
<td>0.074</td>
</tr>
<tr>
<td>II and III</td>
<td>17.33650</td>
<td>0.836</td>
</tr>
</tbody>
</table>
Figure 14 shows the IL-18 levels between three groups
**Figure 15** represents the **hs-CRP levels** with regard to the duration of treatment. When mean values were compared between the patients whose duration of treatment was less than six months and more than six months the values has reduced but it was not statistically significant (p=0.188).
Figure 16 represents the IL-6 levels with respect to the duration of treatment. There was no statistical significance (p=0.230) between the patients with less than six months duration of treatment and more than six months of treatment but their mean values have reduced.
Figure 17 represents the IL-18 levels with regard to the duration of treatment. There was a reduction in the mean values between the less than six months and more than six months treatment but there was no statistical significance between them (p=0.441).
Figure 18 represents the IL-6 levels
Figure 19 represents the IL-18 levels
Figure 20 represents the hs-CRP levels
DISCUSSION

This study was intended to evaluate the status of inflammation, by measuring hs-CRP, IL-6 and IL-18, in PCOS patients in Indian population. It was also aimed to assess the influence of drugs prescribed in PCOS patients on the inflammation.

Polycystic Ovary Syndrome is an endocrine disorder which is very common in the female population in the reproductive age group\textsuperscript{24}. Most women shows the features of metabolic syndrome. It is also concomitant with hypertension, diabetes, dyslipidaemia, gestational type 2 diabetes\textsuperscript{154}.

PCOS is associated with obesity and also increased incidence of insulin resistance\textsuperscript{155}. There is also increased risk of cardiovascular complications. The inflammation which is of chronic low grade is related to PCOS. The C-reactive protein levels is an acute phase reactant and is circulating marker of cardiovascular events. CRP > 3mg/L in a patient is equal for metabolic syndrome as in ATP III guidelines. IL-6, TNF-\(\alpha\), IL-18 concentrations in serum are the predictors of cardiovascular events\textsuperscript{3}. Hence, in this study we proposed to measure the IL-6, IL-18 and hs-CRP levels in PCOS patients in Indian population. There are various studies showing the significance of IL-6, IL-18 and
hs-CRP in PCOS in other countries. There are no studies showing this significance in Indian Population.

There were many therapies of polycystic ovarian syndrome include clomiphene citrate, insulin sensitizing agents, gonadotropins and gonadotropin- releasing hormone (GnRH) analogs, laparoscopic ovarian drilling and the application of assisted reproduction techniques. Apart from this weight loss, diet and exercise have been shown to improve hyperinsulinemia, menstrual abnormalities and ovulation rates\textsuperscript{156}. In previous studies drugs like metformin and thiazolidinones like pioglitazone, rosiglitazone decreases insulin resistance and also known to decrease the inflammation. Other drugs like myo-inositol, clomiphene citrate improves ovulation alone. Their role in inflammation was not known in PCOS.

Ninety participants were recruited in this study. These participants were divided into three groups as normal controls, newly diagnosed patients and already diagnosed on treatment patients. The age group in normal controls was 40\% in 3\textsuperscript{rd} decade and 60\% in 4\textsuperscript{th} decade. The age group in newly diagnosed was 73.3\% and already diagnosed was 66.67\% in the 3\textsuperscript{rd} decade whereas in 4\textsuperscript{th} decade it was 13.33\% and 26.67\% respectively. In normal controls there were no patients in <20 years of
age whereas in newly diagnosed and already diagnosed on treatment groups 13.33% and 6.67% were under 20 years of age respectively and no patients in the age of more than 40 in all the three groups (Figure 1).

On comparing patients’ weight, there were 53.33% in normal controls, 43.33% in newly diagnosed and 50% in already on treatment groups have Normal weight. Overweight was 30%, 36.67%, 33.33% in Normal controls, newly diagnosed, already on treatment groups respectively. 13.33%, 3.33%, 13.33% participants were obese in normal controls, newly diagnosed, already on treatment groups. Only 3.33% were underweight in both normal and already diagnosed on treatment group participants whereas in newly diagnosed there were 16.67% (Figure 2).

In newly diagnosed and already diagnosed on treatment group, participants attended outpatient department of Obstetrics and Gynaecology for both menstrual abnormalities either amenorrhoea or oligomenorrhoea and Infertility. Out of thirty participants in newly diagnosed PCOS, 66.67% and 33.33% attended the outpatient for menstrual abnormalities and for infertility. In already diagnosed patients 60% and 40% attended due to menstrual abnormalities and infertility. Thus participants in both the groups attended the outpatient department mostly for oligo/amenorrhoea (Figure 3). In newly diagnosed patients
60% of the patients were married and in already diagnosed patients 80% of the patients were married. In married patients most of the patients came with the history of infertility (Figure 4).

Only 6.67% in newly diagnosed and 6.67% in already diagnosed patients were hypothyroid and they were on treatment on levothyroxine. Seven patients (23.33%) in newly diagnosed and six patients (20%) in already diagnosed patients’ level of thyroid was not found. Remaining patients were normal in both the groups (70% and 73%) (Fig. 5 & 6).

In case of prolactin levels, in newly diagnosed fifteen patients (50%) have normal levels and for another fifteen patients (50%) levels were not found. In already diagnosed on treatment group it was normal for eleven patients (36%) and abnormal for 2 patients (6.67%). It was not checked for seventeen patients (56.6%) (Fig. 7 & 8).

Low grade chronic inflammation is associated with Polycystic Ovary Syndrome and it can be assessed using inflammatory markers like IL-6, IL-18 and hs-CRP. These are strong predictors of cardiovascular complications. An article proposed by Samy et al compared PCOS patients with BMI matched controls and reported that there is a significant increase in hs-CRP, IL-6 and TNF-α only in the obese group of patients. They have also showed a positive correlation between these
inflammatory markers and HOMA index which was used to measure the insulin resistance\textsuperscript{157}. Kelly et al. reported a study exhibiting an increase in CRP which shows a low grade chronic inflammation in PCOS patients. In this study they showed mean value of PCOS patients was greater than normal controls\textsuperscript{3}.

Here in this study there was no significant increase in the CRP levels (p=0.975). There was no significant difference between the newly diagnosed and already diagnosed patients suggesting that hs-CRP didn’t rise significantly in already diagnosed group as they were on treatment (Table 3 & 4) (Figure 13).

In the same way there was also no significant changes in the IL-6 levels (p=0.746). There was an increase in their mean values when compared to normal controls. Mean value for normal controls was 34.35±93.88 whereas for newly diagnosed patients it was 90.58±308.62 and for already diagnosed it was 91.41±476.6 suggesting there was a rise in IL-6 levels in PCOS when compared to normal controls but that was not significant. Between newly diagnosed patients and already diagnosed patients there was no much difference between the levels (Table 5 &6) (Figure 14).
Another study by Hector et al showed there was an increase in IL-18 independent of obesity. Here in this study the results showed there was an increase in the mean values of IL-18 in PCOS patients when compared to normal controls. The mean value for Normal control was $131.4\pm 224.17$ whereas for newly diagnosed and already diagnosed group of patients it was $264.86\pm 316.33$ and $282.2\pm 403.92$ respectively. The difference was not statistically significant ($p=0.148$) (Table 7&8) (Figure 14). There was also, as in IL-6, no difference in newly diagnosed and already diagnosed patients.

When we considered the treatment prescribed to the patients in already diagnosed group, most of the patients were in Metformin combination (Table 1). Nearly 70% of patients were in metformin combination (Figure 9). Metformin was given along with myoinositol, clomiphene citrate, Human Chorionic Gonadotropins (HCG). Only 46.67% patients were on monotherapy of metformin (Table 2).

The other common drug prescribed was myoinositol combination. It was around 33.33% patients who were prescribed with this combination. It was also given along with metformin, clomiphene citrate and HCG. Metformin along with myoinositol was given to 13.3% of patients.
Menstrual abnormality was one of the chief complaints of the patient (60%) in this group. So they were given low dose of medroxyprogesterone, ethinylestradiol, Cyproterone acetate for about seven patients (23.3%). As patients come for infertility (40%) they were given clomiphene citrate (20%) and HCG (13.3%) injection. Laparoscopic Ovarian Drilling was done for one patient out of the thirty patient and she was prescribed with myooinositol and HCG injection later.

86.67% of patients were on these treatment for less than 1 year and only 13.33% of patients were on these therapies for more than 1 year (Figure 11).

Mekkawi et al stated that there was a statistical significant change in the Interleukin-6 and Interleukin-18 levels before and after metformin therapy showing metformin decreases the inflammatory markers in PCOS patients\(^7\). In previous studies it was known that there was an increase in the Nuclear Factor κ B (NFκB) in PCOS. Metformin inhibits NFκB which inhibits IL-1β and inhibits the production of proinflammatory gene\(^7\).

In this study, the mean ± SD for hs-CRP, IL-6,IL-18 were decreased between the patients who were on treatment for less than six months and more than six months (Figure 15,16,17). But there was no statistical
significance in the hs-CRP (p=0.188), IL-6 (p=0.230) and IL-18 (p=0.441) between these two group of patients. We could also see a decrease in the mean values of hs-CRP, IL-6 and IL-18 as there was an increase in the duration of treatment (Figure 18, 19, 20).

Our results were consistent with Mohlig et al. who showed there was no substantial decrease in CRP and IL-6 levels. In their study these levels were increased in both normal and PCOS obese patients compared to the lean patients. There was a positive relationship between the obesity, insulin resistance and inflammatory markers than the PCOS itself. When a linear regression was done in their study it showed obesity was the one which increases the inflammatory marker than the insulin resistance. Another study by Escobar-Morreale et al. also showed obesity causes an increase in the inflammatory marker and not the disease itself.

In this study we had only 13.3% of obese study population in the already diagnosed and on treatment group and 3.33% in the newly diagnosed PCOS group. Also in this study there were about 86.7% of already diagnosed group of patients’ on treatment for less than one year. So even though there was a fall in the mean values of inflammatory markers there was no statistical significance in them.
The lack of statistical significance may be due to small sample size in each group and wide range of values between the patients. The duration of treatment which was less than one year in most of the patients and hence the effect of drugs on the inflammatory markers could not be assessed. As this was a cross sectional study and blood samples were taken at one time the difference in the inflammatory markers could not be studied for the same group of patients. The influence of drugs prescribed were also in combination for most of the patients and so they could not be studied individually.
SUMMARY & CONCLUSIONS:

Though there are various pharmacological drugs available to treat PCOS, only few drugs targeted in reduction of inflammation. There were various studies, as we quoted earlier, demonstrating the effect of inflammation on PCOS. These studies were all done in other parts of the world. There were very less number of studies done in India. So we proposed to study the status of inflammation on PCOS in Indian population in particular South Indian population.

The pro inflammatory cytokines were produced when NFκB was stimulated. Metformin inhibits this NFκB and thus reduces the inflammation. Other drugs used in PCOS in practise like myoinositol, clomiphene citrate, hormones target at the disease per se and not on the inflammation produced by the disease.

This study has shown that mean values of inflammatory markers were increased in Polycystic Ovary Syndrome (PCOS) in newly diagnosed and already on treatment for PCOS. 86.7% of the patients who were on already treatment for PCOS group were on treatment for less than one year. It has shown that there was an inversely proportional relationship between the mean values of inflammatory markers and duration of treatment.
We suggest larger follow up studies with longer duration of treatment so that influence of drugs on the inflammatory markers can be studied exhaustively in Indian population. Further studies are needed to demonstrate the effects of drugs for PCOS targeting on the inflammatory pathway so that these drugs not only brings down the severity of PCOS but also reduces the cardiovascular risk factors associated with it.
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### ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>Ab</td>
<td>Antibody</td>
</tr>
<tr>
<td>AMPK</td>
<td>Adenosine Monophosphate Kinase</td>
</tr>
<tr>
<td>AGE</td>
<td>Advanced Glycated End products</td>
</tr>
<tr>
<td>ASRM</td>
<td>American Society of Reproductive Medicine</td>
</tr>
<tr>
<td>CC</td>
<td>Clomiphene Citrate</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
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<tr>
<td>FSH</td>
<td>Follicle Stimulating Hormone</td>
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<tr>
<td>GnRH</td>
<td>Gonadotropin Releasing Hormone</td>
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<tr>
<td>GnRHg</td>
<td>Gonadotropin Releasing Hormone agonist</td>
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<tr>
<td>hs-CRP</td>
<td>High sensitivity C-reactive protein</td>
</tr>
<tr>
<td>HRP</td>
<td>Horse Radish peroxidase</td>
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<tr>
<td>IL-6</td>
<td>Interleukin-6</td>
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<tr>
<td>IL-18</td>
<td>Interleukin-18</td>
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<tr>
<td>IL-8</td>
<td>Interleukin-8</td>
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<tr>
<td>IL-1β</td>
<td>Interleukin-1β</td>
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<tr>
<td>IR</td>
<td>Insulin Resistance</td>
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<tr>
<td>IVF</td>
<td>In Vitro Fertilization</td>
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<tr>
<td>LH</td>
<td>Luteinizing Hormone</td>
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<tr>
<td>NAC</td>
<td>N-Acetyl Cysteine</td>
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<tr>
<td>NFκB</td>
<td>Nuclear Factor kappa B</td>
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PCOS – Polycystic Ovary Syndrome
ROS – Reactive Oxygen Species
Std. – Standard
CASE REPORT FORM

NAME :

AGE :

O.P. NUMBER :

HEIGHT :

WEIGHT:

HISTORY:

DRUG HISTORY:

USG FINDINGS:

INTERLEUKIN-6:

INTERLEUKIN-18:

hs CRP:

FSH :LH (if done) :
I, Senthur Selvi R., am carrying out a study on the topic: A CROSS SECTIONAL STUDY ON THE STATUS OF INFLAMMATORY MARKERS IN POLYCYSTIC OVARY SYNDROME (PCOS) PATIENTS IN INDIAN POPULATION as part of my research project being carried out under the aegis of the Department of PHARMACOLOGY.

My research guide is: Dr. K. BHUVANESWARI, Professor and Head of the department, Pharmacology.

The justification for this study is: PCOS is prevalent nowadays. There are no studies demonstrating the role of inflammation in patients with PCOS in Indian population and also how the drugs prescribed for PCOS influences the inflammation.

The objectives of this study are:

Primary Objective: To assess the status of inflammation in patients with PCOS

Secondary Objective: to assess the influence of drugs prescribed for PCOS on inflammation

Sample size: 90.

Study volunteers / participants are (specify population group & age group): Patients diagnosed to have PCOS and also healthy volunteers with age group between 18-45 years.

Location: The study will be conducted in PSGIMS&R hospital.

We request you to kindly cooperate with us in this study. We propose collect background information and other relevant details related to this study. We will be carrying out:

Initial interview (specify approximate duration): 20 minutes.

Data collected will be stored for a period of five years. We will not use the data as part of another study.

Health education sessions: Number of sessions: one. Approximate duration of each session: 20 minutes.

Clinical examination (Specify details and purpose): Height and Weight for BMI

Blood sample collection: Specify quantity of blood being drawn: 5 ml.

No. of times it will be collected: 1

Whether blood sample collection is part of routine procedure or for research (study) purpose:
Research purpose

Specify **purpose**, discomfort likely to be felt and side effects, if any: pain while pricking

Whether blood sample collected will be stored after study period: No, it will be destroyed

Whether blood sample collected will be sold: No

Whether blood sample collected will be shared with persons from another institution: No

**Medication** given, if any, duration, side effects, purpose, benefits: No

**Benefits** from this study: PCOS is prevalent nowadays. There are no studies demonstrating the role of inflammation in patients with PCOS in Indian population and also how the drugs prescribed for PCOS influences the inflammation.

**Risks** involved by participating in this study: No risks

How the **results** will be used: Clinical meeting at PSG Hospitals
Dr. MGR Medical University dissertation

If you are uncomfortable in answering any of our questions during the course of the interview / biological sample collection, **you have the right to withdraw from the interview / study at anytime**. You have the freedom to withdraw from the study at any point of time. Kindly be assured that your refusal to participate or withdrawal at any stage, if you so decide, will not result in any form of compromise or discrimination in the services offered nor would it attract any penalty. You will continue to have access to the regular services offered to a patient. You will **NOT** be paid any remuneration for the time you spend with us for this interview / study. The information provided by you will be kept in strict confidence. Under no circumstances shall we reveal the identity of the respondent or their families to anyone. The information that we collect shall be used for approved research purposes only. You will be informed about any significant new findings - including adverse events, if any, – whether directly related to you or to other participants of this study, developed during the course of this research which may relate to your willingness to continue participation.

**Consent:** The above information regarding the study, has been read by me/ read to me, and has been explained to me by the investigator/s. Having understood the same, I hereby give my consent to them to interview me. I am affixing my signature / left thumb impression to indicate my consent and willingness to participate in this study (i.e., willingly abide by the project requirements).

Signature / Left thumb impression of the Study Volunteer / Legal Representative:

Signature of the Interviewer with date: Witness:

Contact number of PI:

Contact number of Ethics Committee Office: 0422 2570170 Extn.: 5818
அப்போ பாலம்

புகழ்:

இண்டு சந்திக்க உலகில் ஆண்ட பொருள், PSG உற்பத்திய கல்வியான பாதுகாப்புகள் குறிப்பிட்டு, இடைய்விளையான பாதுகாப்புகள் தொடங்கியுள்ளது. இடைய்விளையான பாதுகாப்பு அமைப்பின் நூற்றாண்டில் அமைந்த நூற்றாண்டில் அமைந்த நூற்றாண்டில் அமைந்த நூற்றாண்டில் அமைந்த நூற்றாண்டில் அமைந்த நூற்றாண்டில் அமைந்த நூற்றாண்டில் அமைந்த நூற்றாண்டில்

கோவில் சோகணை: K. பிளாஸ்யான்கள், சக்கரயான பல்கலைக் கழகம்

அப்போ சிதையாறானவர் அப்போடு : பல காலங்களால் இடைய்விளையான பாதுகாப்பு நூற்றாண்டில் இடைய்விளையான பாதுகாப்பு நூற்றாண்டில் அமைந்த நூற்றாண்டில் அமைந்த நூற்றாண்டில் அமைந்த நூற்றாண்டில் அமைந்த நூற்றாண்டில் அமைந்த நூற்றாண்டில் அமைந்த நூற்றாண்டில்

ஆப்போ பொதுவாக்கம்: ரீது போக்கினால் ஆப்போ பொதுவாக்கம் நூற்றாண்டில் அமைந்த நூற்றாண்டில் அமைந்த நூற்றாண்டில் அமைந்த நூற்றாண்டில்

ஆப்போ பாலாக்கத்தவர் பல்கலைக் கழகத்தின்: 90%

ஆப்போ சிதையாறானவர் பல்கலைக் கழகத்தின்: PSG உற்பத்தியாளர்

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- 2 -
அப்பாளரின் எண்ணிக்கை / பக்த விளக்கப்பட்டு: எல்லைப்பிள்ளை.

இன்று அவர்கள் இடத்திலும் துளர்ந்து 5 லட்சம்பாவும் புறநிலைக்கோளை விளக்கும். இவ்வுறுத்துதல் அவர்கள் பல்லவர் உயர்ந்து மாற்றம் பூச்சியத்திற்கு மாற்றம் பெற்றுத் தொடர்ந்து புணர்பெருக்கம் மாற்றம் பெற்றுத் தொடர்ந்து கொண்டுவருகின்றனர். இன்று அவர்கள் பல்லவரை உயர்ந்து மாற்றம் பூச்சியத்திற்கு மாற்றம் பெற்றுத் தொடர்ந்து புணர்பெருக்கம் மாற்றம் பெற்றுத் தொடர்ந்து கொண்டுவருகின்றனர்.

இன்று அவர்களின் காரணத்தால் சிறுவர்கள் விளக்கப்படும் / கூட சிறுவர் விளக்கப்படும் உண்மை என்ற விளக்கத்தை விளக்கப்படும்.

அப்பாளரின் காரணமாக:

வெறும்:

அப்பாளரின் வெறும்:

இன்று அவர்களின் உயர்ந்து மாற்றம் அறிவு பல்லவர் உயர்ந்து மாற்றம், விளக்காற்றுதல் உயர்ந்து மாற்றம் பெற்று உயர்ந்து மாற்றம் பெற்று உயர்ந்து மாற்றம் பெற்று உயர்ந்து மாற்றம் பெற்று உயர்ந்து மாற்றம் பெற்று.

அப்பாளரின் ரீதியாக, இயக்கு

காரணமாக:

சிறுவர்:

அப்பாளரின் விளக்கப்படும் லேன்:

லேன் உரையான கால் அவர்களின் விளக்கப்பட்டு: 0422 2570170 Extn: 5818
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Clinical examination (Specify details and purpose): Height and Weight for BMI

Blood sample collection: Specify quantity of blood being drawn: 5 ml.

No. of times it will be collected: 1

Whether blood sample collection is part of routine procedure or for research (study) purpose:
Research purpose

Specify purpose, discomfort likely to be felt and side effects, if any: pain while pricking

Whether blood sample collected will be stored after study period: No, it will be destroyed

Whether blood sample collected will be sold: No

Whether blood sample collected will be shared with persons from another institution: No

Medication given, if any, duration, side effects, purpose, benefits: No

Benefits from this study: PCOS is prevalent nowadays. There are no studies demonstrating the role of inflammation in patients with PCOS in Indian population and also how the drugs prescribed for PCOS influences the inflammation.

Risks involved by participating in this study: No risks

How the results will be used: Clinical meeting at PSG Hospitals
Dr.MGR Medical University dissertation

If you are uncomfortable in answering any of our questions during the course of the interview / biological sample collection, you have the right to withdraw from the interview / study at anytime. You have the freedom to withdraw from the study at any point of time. Kindly be assured that your refusal to participate or withdrawal at any stage, if you so decide, will not result in any form of compromise or discrimination in the services offered nor would it attract any penalty. You will continue to have access to the regular services offered to a patient. You will NOT be paid any remuneration for the time you spend with us for this interview / study. The information provided by you will be kept in strict confidence. Under no circumstances shall we reveal the identity of the respondent or their families to anyone. The information that we collect shall be used for approved research purposes only. You will be participating in this study as a healthy volunteer to assess the normal limits. You will be informed about any significant new findings - including adverse events, if any, – whether directly related to you or to other participants of this study, developed during the course of this research which may relate to your willingness to continue participation.

Consent: The above information regarding the study, has been read by me/ read to me, and has been explained to me by the investigator/s. Having understood the same, I hereby give my consent to them to interview me. I am affixing my signature / left thumb impression to indicate my consent and willingness to participate in this study (i.e., willingly abide by the project requirements).

Signature / Left thumb impression of the Study Volunteer / Legal Representative:

Signature of the Interviewer with date: Witness:

Contact number of PI:
Contact number of Ethics Committee Office: 0422 2570170 Extn.: 5818