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"EVALUATION OF HOMOCYSTEINE LEVELS AND ITS CORRELATION WITH CLINICAL, METABOLIC AND HORMONAL PROFILE OF WOMEN WITH PCOS"

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Abstract:

Background: PCOS is the most common endocrinological disorder affecting 6 -14% amongst reproductive age group. The pathophysiology of PCOS is complex and it is still not well understood. Many markers have been studied to correlate between homocysteine level and other clinical, metabolic and hormonal profile. Preliminary investigations indicate that in women with PCOS, serum biomarkers of cardiovascular disorders such as homocysteine are abnormal. Furthermore the interactions between homocysteine and PCOS, biochemical features such as obesity, insulin resistance and higher levels of androgen, have been researched and the potential determinant of this finding is still being explored.

Aim and Objectives: We aim to determine association between hyperhomocysteinemia and PCOS. The objectives of this study is to evaluate and compare clinical, metabolic and

hormonal profile in women of PCOS with normal homocysteine level with that of increased homocysteine level.

Materials and Methods: This is a prospective observational study which will be performed in the department of obstetrics and gynaecology, AVBRH, DMIMS (Deemed to be University, Wardha), a tertiary care teaching hospital situated in the rural area of Wardha district in over 140 patients for period between 2020-22. This study will include PCOS patients based on Rotterdam diagnostic criteria and will exclude patients with known metabolic, cardiovascular and endocrine disorders. Metabolic and hormonal profile will be evaluated with BMI, Lipid profile, Insulin resistance, serum levels of LH, FSH, DHEA and Testosterone in all patients. Serum homocysteine levels will be measured using ELISA.

Expected Results: We expect that there will be significant difference in clinical, metabolic and hormonal profile of PCOS women having hyperhomocysteinemia than women having normal homocysteine level.

Keywords: PCOS, Homocysteine, Rotterdam Criteria, BMI, Lipid profile, FSH, LH, DHEA, Testosterone

INTRODUCTION

The most common endocrine abnormality is polycystic ovarian syndrome (PCOS), affecting 6to14 percent of women in the reproductive age group. (1) Ovulation disorders, hyperandroge nism, inc-eased abortion rate, and infertility are induced.
Some conditions related to polycystic ovarian syndrome include-
hyperinsulinemia, obesity, dyslipidemia, diabetes, hypertension, atherosclerosis and vascular diseases.

Polycystic ovarian syndrome is a complex and heterogeneous endocrinological disorder characterised by a mixture of clinical features and symptoms, including polycystic ovarian morphology, ovarian dysfunction (menstrual irregularities) and hyperandrogenism (clinical or biochemical). There are currently many diagnostic criteria that use different constellations of these clinical traits for polycystic ovarian syndrome (2)

The diagnosis of polycystic ovarian syndrome is based on the criteria of Rotterdam, which include the existence of two of the three findings: ovulatory dysfunction, hyperandrogenism and polycystic ovaries, plus the exclusion of other diagnoses that could contribute to ovulatory dysfunction or hyperandrogenism. Evaluation of adolescent patients for polycystic ovary syndrome is appropriate to wait until two years after menarche. All three Rotterdam requirements are also recommended to be met before a diagnosis is made for this age group.

The sulphur-containing amino acid homocysteine is synthesised from methionine. Once formed, through a reversible reaction that requires folate and B12, it can be methylated back into methionine. Concentrations higher than 12.0 μmol/L are commonly defined as hyperhomocysteinemia. The mechanisms by which homocysteine triggers the development and progression of cardiovascular diseases have not been fully described. However, current research shows the importance of homocysteine as the culprit for endothelial damage and dysfunction.

Preliminary evidence indicates that in women with polycystic ovarian syndrome, there are pathological serum biomarkers of cardiovascular disorders, such as homocysteine, adiponectin and C-reactive high-sensitivity protein.(3)

Increased insulin levels are postulated to increase homocysteine levels by inhibiting the function of hepatic cystathionine synthase, which is a characteristic of insulin resistance. Via increased synthesis of monocyte chemoattractant protein-1 and interleukin-8 expression and secretion, homocysteine promotes leukocyte recruitment. Homocysteine stimulates the development of collagen and increases the proliferation of smooth muscle cells. Homocysteine's prothrombotic effects include reduced plasminogen activator binding sites in endothelial cell tissue, heparin sulphate, protein C inhibition and factor VIIa and V activation, increased blood viscosity, increased prothrombin fragments 1 and 2, fibrinopeptide A and decreased endothelial anti-thrombotic activity due to derangement of thrombomodulin function (4)

Past studies have investigated the link between PCOS and homocysteine with conflicting findings, given the association between insulin resistance and polycystic ovarian syndrome and also between hyperinsulinemia and elevated plasma homocysteine levels. In poly cystic ovarian syndrome patients, hyperandrogenemia has also been shown to be associated with hyperhomocysteinemia.

Homocysteine levels have been postulated to be higher in patients with poly cystic ovarian syndrome relative to controls and are portrayed as an independent risk factor for cardiac and vascular diseases.

Many studies have been performed over the last decade to examine serum homocysteine levels in women with polycystic ovarian syndrome; in addition, the associations between polycystic ovarian syndrome and homocysteine, biochemical features (such as obesity, insulin resistance and raised androgen levels) have also been investigated(1) and the potential determinants of this finding have been studied.(1) We therefore seek to assess the levels of homocysteine and its correlation with the clinical, metabolic and hormonal profile of women with polycystic ovarian syndrome" in this study.”

AIM AND OBJECTIVES:

AIM

We aim to study and compare the clinical, metabolic and hormonal profile in polycystic ovarian syndrome women of reproductive age group with normal and elevated homocysteine level to find out any association of hyperhomocysteinemia with these profiles in PCOS. If this corelation is proven then we can recommend screening for hyperhomocystinemia and treat if found in PCOS women with anticipated benefits.

OBJECTIVES

1. To study the clinical, metabolic and hormonal profile in women of PCOS with normal homocysteine level.

2. To study the clinical, metabolic and hormonal profile in women of PCOS with increased homocysteine level

3. Comparison between above two groups.

MATERIALS AND METHODS:

Study Design-prospective observational study

Place of study -department of OBGY, AVBRH, DMIMS (Deemed to be University, Wardha)

Duration of study – 2020-2022

Sample size with desired population:

$$\frac{Z_{x/2(sq)} \times Px(1-P)}{d(sq)}$$

Where:

Z x/2 = is the level of significance at 5% that is 95% confidence interval = **1.96**

P= prevalence of PCOS = 6% = **0.06**

d= desired error of margin= 4% = **0.04**

So, **n =**

$$\frac{1.96*1.96*0.06*(1-0.06)}{0.04*0.04}$$

= 135.41

N = minimum **140** patients

INCLUSION CRITERIA

1. All cases with confirmed diagnosis of PCOS based on the Rotterdam diagnostic criteria.
2. All eligible women giving informed written consent.

EXCLUSION CRITERIA

1.Women not willing to participate.

2.Women with known metabolic diseases.

3.Women with known cardiovascular diseases.

4.Women with other endocrine disorder.

5.Women with other causes of Hyperhomocysteinemia -

a) Renal failure

b) Liver disease

c) Smokers

METHODOLOGY

The present observational prospective study will be conducted in the Department of Obstetrics & Gynaecology, AVBRH, DMIMS(DU), Sawangi (Meghe), Wardha in over 140 PCOS women.

Ethical clearance from the institutional ethical committee will be taken.

ENROLMENT

DIAGNOSIS OF PCOS

It will be done as per ROTTERDAM criteria: Oligo/amenorrhea, anovulation, ultrasonic polycystic ovaries, clinical and/or biochemical symptoms of unexplained hyperandrogenism, hirsutism/acne/hoarseness or speech shifts.

Before enrolling the patient into the study, every woman will be explained the type and nature of study and informed consent will be taken. The women of reproductive age group will be selected as participants.

1. Demographic data like Age, parity, rural/ urban history, history of previous pregnancies including abortions, obstetric history, menstrual history along with relevant medical history will be recorded on predesigned & pretested proforma.
2. Physical and pelvic examinations, laboratory findings will be noted. Routine investigations like complete blood counts, blood grouping, liver function test, kidney function test, urine examination, viral markers will be done.
3. Serum homocysteine level will be done. Fasting blood samples will be collected for the measurement of serum homocysteine concentration in EDTA vacutainer tubes and transported to the laboratory within 30 mins. Serum homocysteine (micromole/L) levels will be measured using the Enzyme- Linked immunosorbent Assay.
4. This investigation is a part of basic hormonal profile for PCOS. Serum homocysteine level is done in our AVBRH Hospital.
5. We shall be applying for funding from intramural grant/ICMR grant/concession for project cost.
6. Women with normal homocysteine level will be categorised into **group A** and those women with increased homocysteine level will be categorised into **group B.**

OUTCOMES :

The Parameters of clinical, metabolic and hormonal profile will be studied and compared between A and B group.

1)Clinical Profile -

- According to ROTTERDAM criteria

a) Oligo/amenorrhea
b) Anovulation
c) Ultrasonic polycystic ovaries

d) Clinical and/ or biochemical signs of unexplained hyperandrogenism: hirsutism / hoarseness / acne or speech shift

2)Metabolic Profile – a) BMI= weight*weight/height

b) Lipid profile suggesting dyslipidaemia, HDL, LDL, VLDL, total cholesterol.

c) Insulin resistance with fasting blood sugar (FBS)

3)Hormonal Profile - will be measured at day 3 of menstrual cycle

a) FSH

b) LH

c) Testosterone level

d) DHEA

Final comparison and correlation of various outcomes in two different groups will be assessed using statistical method: Chi-square test and Student's unpaired t test and results would be obtained.

Statistical method: Chi-square test and Student's unpaired t test

EXPECTED OUTCOME:

There will be significant difference in clinical, metabolic and hormonal profile of PCOS women having hyperhomocysteinemia than women having normal homocysteine level.

Clinical – PCOS women with hyperhomocysteinimia will have significantly more incidence of clinical features of hyperestrogenism, hyperandrogenism and insulin resistance than with normal homocysteine level.

Metabolic- PCOS women with hyperhomocysteinemia will have higher BMI, dyslipidemia and higher fasting blood sugar than with normal homocysteine level.

Hormonal -PCOS women with hyperhomocysteinemia will have significantly abnormal FSH, LH and testosterone level than with normal homocysteine level.

Serum homocysteine levels can then be included as routine investigation in women with PCOS.

DISCUSSION:

Present study is aimed to compare the clinical, metabolic and hormonal profile in women of the reproductive age group with polycystic ovarian syndrome, having normal and elevated homocysteine level.

Dysregulation of the levels of a variety of biochemical markers (including branched chain amino acids, leptin and adiponectin) has been suggested to assist in the production and

maintenance of the phenotype of polycystic ovarian syndrome. (5) In addition to insulin resistance (IR) and above markers, serum homocysteine levels are studied recently in women with polycystic ovarian syndrome to compare their effect and association with phenotypes and clinic, biochemical profiles of these women.

The findings of the different studies are currently not definitive in relation to the correlation between the amount of homocysteine and the clinical, metabolic and hormonal profile of polycystic ovarian syndrome. Studies to determine the relationship of hyperhomocysteinemia with the phenotype of polycystic ovarian syndrome and the biochemical profile would be useful in the treatment and prevention of the disorder or its co-morbidities (5).

The studies currently conducted that are correlated with the clinical, metabolic and hormonal profile are sparse. Higher levels of homocysteine were shown to be detectable in women with polycystic ovarian syndrome irrespective of body weight status. Whether increased homocysteine levels are predisposed to PCOS or PCOS results in an increase in the amount of serum homocysteine is difficult to state. The association of elevated homocysteine serum levels in women with polycystic ovarian syndrome is significant because of the negative impact of elevated homocysteine levels on the cardiovascular system. It is well known that patients with polycystic ovarian syndrome are more likely to develop cardiovascular complications, so that improvements in the level of homocysteine in these patients may play an important role in or at least in the progression map of this syndrome's pathogenesis.

The biochemical characteristic of poly cystic ovarian syndrome is hyperandrogenemia. Synergistically, elevated levels of luteinizing hormone and insulin improve androgen synthesis. In about 60 percent of cases, poly cystic ovarian syndrome is a common cause of hirsutism, although this varies according to race and degree of obesity (6)

The probable reason for this lipid profile imbalances found in patients with poly cystic ovarian syndrome could be accredited to raise levels of androgens associated with poly cystic ovarian syndrome pathogenesis and this higher level of androgens contributes to a weakening of the sensitivity of the insulin receptor leading to metabolic derangement with its atherogenic potential This is supported by the belief that lipid parameters seen in poly cystic ovarian syndrome typically have an unresolving impact and are related to insulin resistance. With a rise in BMI and waist circumference, the increase in homocysteine in poly cystic ovarian syndrome was more noticeable (4)

A recent study found that higher homocysteine was correlated with sporadic anovulation and hormonal changes that may be suggestive of impaired ovulatory activity. (7).

The findings of this research are expected to be consistent with previous studies that found that a characteristic finding that is observed in women with poly cystic ovarian syndrome is a rise in body weight.

Some studies have shown that in women with insulin-resistant poly cystic ovarian syndrome, mean plasma homocysteine levels are significantly elevated relative to women with non-insulin-resistant poly cystic ovarian syndrome irrespective of BMI, which suggests a link between plasma homocysteine and plasma insulin levels. Hyperhomocysteinaemia is exaggerated by all those associated with the metabolic syndrome. The risk factor for cardiovascular disorders, dyslipidemia, hypertensive nephropathy and diabetes is insulin resistance. Polycystic ovarian syndrome could then be treated as a form of syndrome of insulin resistance or as an early symptom of that syndrome. It should however be held in mind that many factors influence the amount of homocysteine plasma. The importance of

considering plasma homocysteine levels and its hostile role in the metabolic complications of patients with polycystic ovarian syndrome was further emphasised in these findings (8)

In accordance with Vrbikova et al., we expect to see a substantial difference and positive association of homocysteine with androstenedione and resistance to homocysteine and insulin. Therefore, hyperhomocysteinemia may be directly linked to atherogenesis, so high homocysteine levels can help to recognise a subgroup of women who are at risk of developing polycystic ovarian syndrome(9). The evidences of this problem are available through many GBD studies(10,11,12). Few of the studies related to similar issues were reported(13,14,15).

CONCLUSION:

There will be significant difference in clinical, metabolic and hormonal profile of polycystic ovarian syndrome women having normal and abnormal homocysteine levels.

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