Serum homocysteine level after laparoscopic ovarian drilling in women with polycystic ovary syndrome

Thesis

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By

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Aim of the work

The aim of this work is to evaluate the serum homocystiene level before and three months after laparoscopic ovarian drilling in patients with polycystic ovary syndrome.

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List of abbreviations

ART = Assisted reproductive technique
BMI = Body mass index
CAD = Coronary artery disease
CC = Clomephene Citrate
CCR = Clomephene Citrate resistance
CPA = Cyproterone acetate
DHEA = Dehydroepiandrostenidione
DHEAs = Dehydroepiandrostenidione sulfate
DSG = Desogestrel
EE = Ethinyl estradiol
E2 = Estradiol
FAI = Free androgen index
FSH = Follicular stimulating hormone
FDA = Food and drug administration
GCP = General cystic pattern
GnRH = Gonadotrophin releasing hormone
GnSAF/GnSIF = Gonadotropin surge attenuating/inhibitory factor
Hcy = Homocystiene
HCG = Human chorionic gonadotrophins
HDL = High density lipoprotein
HPG = Human pituitary gonadotrophin
HMG = Human menopausal gonadotrophin
IGF1 = Insulin like growth factor -1
IR = Insulin resistance
IRS = Insulin resistance syndrome
IVF = In vitro fertilization
Kg = Kilo gram
LDL = Low density lipoprotein
LOD = Laparoscopic ovary diathermy
LH = Luteinizing hormone
MRI = Magnetic resonance imaging
MTX = Methotrexate
MTHFR = Methyltetrahydrofolate reductase
NAC = N-acetyl cysteine
NIDDM = Non-insulin-dependent diabetes mellitus
OCs = Oral contraceptives
OGTT = Oral glucose tolerance test
OHSS = Ovarian hyper stimulation syndrome
PCOS = Polycystic ovary syndrome
PCP = Peripheral cystic pattern
SHBG = Sex hormone binding globulin

S/A = Ovarian stroma/total area
T = Testosterone
USA = United States of America
U/S = Ultrasound
VLCD = Very low calorie diet
WHO = World health organization
W/H = Waist/ hip ratio

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INTRODUCTION

Polycystic ovary syndrome is a very common problem in a variety of clinical manifestations, including amenorrhea, irregular menses, and hirsuitism. Serious consequences of chronic anovulation are infertility and a greater risk for developing carcinoma of the endometrium and perhaps the breast. There is a new appreciation for the role of hyperinsulinemia in this condition and for the clinical effect of hyperinsulinemia and hyperandrogenism on the risks of developing cardiovascular disease and diabetes mellitus. The clinician must recognize the clinical impact of anovulation and undertake therapeutic management of all patients to avoid these unwanted consequences (**Speroff, 2005)**.

We cannot overemphasize the importance of a new attitude toward this common female problem that occurs in approximately 4% - 6% of reproductive age women (**Knochenhauer et al., 1998) (Asuncion et al., 2000).** Concern for anovulation in polycystic ovary is a legitimate component of modern preventive health care.

Infertility associated with PCOS has been attributed to numerous factors, including oligo-anovulation, dysfunctional gonadotrphin secretion, elevated systemic and /or local ovarian androgen levels, and dysfunction of any or several ovarian growth factors and their binding proteins (**Balen and Jacob, 2003**).

Research had focused on systemic and local effects of insulin resistance and its secondary effects-systemic, metabolic, and ovarian. Evidence has increased to indicate that hyperinsulinaemia and / or any or all of the phenotypes of the insulin resistance syndromes in the general population may have various deleterious metabolic effects, including causing an increase of plasma homocysteine **(Meigs et al., 2001)**.

Insulin resistance (IR)(or the IR syndromes, IRS) itself is a risk factor for cardiovascular disease , diabetes , hypertension nephropathy, and dyslipidaemia

(**Goldstein et al., 2001)** ; all of the long term implications of these facts of the "metabolic syndromes" can be aggravated by elevated homocysteine. Thus, in metabolic terms, PCOS may be possibly considered another variant of the IRS, or at the very least be considered an early marker of the IRS. As such , the infertility treatment provider should actively search for signs of metabolic dysfunction, including elevated homocysteine levels, and endeavor to optimize metabolic factors in order to achieve the best results in the short term (reproductive function) and in the long term (cardiovascular and metabolic functions) (**Schachter et al., 2003**).

Chapter I

Polycystic ovary syndrome

1-Historical background

Polycystic ovary syndrome (**PCOS**) is the most common endocrine disorder in women of reproductive age and the most common disorder of ovarian function in premenopausal women (**Slowey, 2001)** It is generally accepted that the prevalence of PCOS is approximately 5-10%, and that of polycystic ovaries alone is 21- 23%. Other features of PCOS are infertility, obesity, insulin resistance, impaired glucose tolerance and type 2 diabetes mellitus, dyslipidaemia, cardiovascular disease and obstructive sleep apnea (**Stankewiez and Norman, 2006)**. And such condition is considered as a reproductive- metabolic disorder **(Chang et al., 1999**).

In 1935, **Ireving Stein** and **Micheal Leventhal** first described a symptom complex associated with anovulation. They described 7 patients (4 of whom were obese) with amenorrhea, hirsutism and enlarged polycystic ovaries. They reported the results of bilateral wedge resection removing one half to three froths of each ovary; all 7 patients resumed regular menses and 2 become pregnant. **Stein** and **Leventhal** developed the wedge resection after they observed that several of their amenorrheic patients menstruated after ovarian biopsies. They reasoned that the thickened tunica was perverting follicles from reaching the surface of the ovary (**Speroff, 2005).**

Acceptance of this syndrome as a singular clinical entity led to a rather rigid approach to this problem for many years. Once those women qualified who had a history of oligomenorrhea, hirsutism and obesity, together with a demonstration of enlarged polycystic ovaries a clinical state now recognized to be characteristic of extreme cases. It is far more useful clinically to avoid the use of eponyms and even the term polycystic ovary syndrome or disease. It is better to consider this problem as one of persistent anovulation with spectrum of etiologies and clinical manifestation that now includes insulin resistance and

hyperinsulinaemia, as well as hyperandrogenaemia. Of course, specific conditions must be pursued and excluded. Such as adrenal hyperplasia, thyroid disease, Cushing's syndrome, hyperprolactinaemia and androgen producing tumors (**Speroff, 2005**).

2-**Definition:**

Since the 1990 national institutes of health sponsored conference on polycystic ovary syndrome, it has become appreciated that the syndrome encompasses a broader spectrum of signs and symptoms of ovarian dysfunction than those defined by the original diagnostic criteria.

The 2003 Rotterdam consensus workshop concluded that polycystic ovary syndrome is a syndrome of ovarian dysfunction along with the cardinal features of hyperandrogenism and polycystic ovary morphology. Polycystic ovary syndrome remains a syndrome and as such no single diagnostic criteria (such as **hyperandrogenism** or **polycystic ovary**) is sufficient for clinical diagnosis. Their clinical manifestations may include menstrual irregularities, signs of androgen excess and obesity. Insulin resistance and elevated serum LH levels are also common features in polycystic ovary syndrome that is associated with increased risk of type II diabetes and cardiovascular events **(Rotterdam PCOS consensus, 2003).**

There are two identified definitions for PCOS; PCOS I (the European view) and PCOS 2 (In North America).

The European view, which encompasses, in addition to the ultrasonographic finding of **PCOS**, one or more of the clinical symptoms or biochemical features (i.e. oligoamenorrhea, hyperandrogenism, obesity, elevated serum testosterone or LH concentrations). Ultrasonographic criteria include; presence of >10 cyst , 2-8mm in diameter scattered either around or through an echo dense , thickened central stroma (**Balen, 1999).**

Balen et al. (1995) believe that ovarian morphology appears to be the most sensitive marker for PCOS compared with the classical endocrine features

of a raised serum LH and/or testosterone concentration which were found in only 39.8% and 47.8% of their patients, respectively.

In North America, the syndrome is denoted by the combination of hyperandrogenism and ovulatory dysfunction, in the absence of non- classical adrenal hyperplasia, without necessarily having to identify the presence of polycystic ovaries by ultrasound **(Dunaif, 1997).** This is because the appearance of polycystic ovaries is considered a sign not a disease **(Slowey, 2001).** Polycystic-appearing ovaries present in 6% to 25% of normal population **(Koivunea et al, 1999**) and in about 67% to 80% of **PCOS** patients **(Fox, 1999).** However, European clinicians consider radiological or surgical evidence of polycystic ovaries integral for the diagnosis **(Slowey, 2001).**

Kondoh et al. (1999) defined polycystic ovary syndrome according to the following criteria of the Japan Society of Obstetrics and Gynecology: (1) Amenorrhea or oligomenorrhea with or without hirsutism, (2) A high plasma LH level associated with a low FSH level and an LH/FSH ratio of >1 and (3) Bilaterally normal or enlarged ovaries with multiple small cysts, as assessed by transvaginal ultrasonography.

Cushing's syndrome and late hyperplasia are excluded. Participants at the National Institute of Health conference supported a definition of PCOS that included three key features; **(1)** Ovulatory dysfunction. **(2)** Evidence of hyperandrogenism by clinical examination or laboratory measurement and **(3**) the absence of other endocrine disorders including non-classical adrenal hyperplasia**,** androgen-secreting tumors, hyperprolactinemia and thyroid dysfunction (**Kim et al., 2000; Mitwally and Casper, 2001)**

Revised diagnostic criteria of polycystic ovary syndrome. Two out of three **(Rotterdam PCOS consensus, 2003);** 1.Oligo- or anovulation. 2. Clinical and/or biochemical signs of hyperandrogenism. 3. Polycystic ovaries. Exclusion of other etiologies (congenital adrenal hyperplasia, androgen-secreting tumors, Cushing's syndrome). It is recognized that women with regular cycles and

hyperandrogenism and/or polycystic ovaries may have the syndrome. It has also recognized that some women with the syndrome will have polycystic ovary without clinical evidence of androgen excess but will display evidence of ovarian dysfunction **(Carmina et al., 2001).**

3-Epidemiology:

The prevalence of PCOS can't be determined with precision because it depends on the definition. A strict research-based definition that relies on endocrine characteristics is associated with a 4-6% prevalence of PCOS (**Haas et al., 2003).** And for the clinical definition using chronic anovulation plus androgen excess, the prevalence of PCOS is 5-10 % of premenopausal females **(De wailly, 1997; Slowey, 2001**).

Polycystic ovary syndrome is the most common cause of anovulatory infertility on women of reproductive age. Its prevalence seems to be higher than expected and classical description of the obese, virilized patients with this syndrome that range from individuals with normal body weight , regular menstrual cycles with ultrasonic picture of polycystic ovary, oligomenorrhea, obesity, hirsuitism and hyperandrogenaemia **(Eftekhar, 2004).**

Where as a definition that is based purely on ultrasonography defined morphologic characteristics is associated with 22% prevalence (**Franks et al., 1998).** For clinical definition used here, chronic anovulation plus androgen excess, the prevalence is probably in the 55% range (**De Wailly et al., 1997; Slowey, 2001).**

The prevalence of **PCOS** is increased significantly with the irregularity of the menstrual cycle, 28% of girls with irregular menstrual cycles, and 45% of oligo-amenorrheic girls **(Van Hoff et al., 2000).**

4-Pathophysiology:

Traditional concepts of **PCOS** as a primarily endocrine condition secondary to aberrations in the hypothalamo-pituitary-ovarian axis manifesting as high luteinising hormone/follicle stimulating hormone ratios, increased androgen production and high oestrone levels from peripheral conversion in adipose tissue of androgens are increasingly being challenged. New developments in research into the pathophysiology of **PCOS** have focused on the role of genetics, insulin resistance and the interrelationships between obesity and ghrelin (a gastric peptide with adipogenic activity) (**Sharma et al., 2005**).

Genetics

The familial pattern and identification of a possible male phenotype in **PCOS** makes it a condition that hypothetically is predominantly genetic in origin. Overall, studies on the genetics of PCOS suggest that a gene or several genes may be associated with PCOS, based on the clustering of **PCOS** in families and studies of theca muscle and adipocytes from women with **PCOS** which have highlighted a unique molecular phenotype (**Sharma et al., 2005**).

Results from some family studies suggest a possible autosomal dominant phenotype. However, the large twin study on women with **PCOS** (**Jahanfar et al., 1995**) found a high degree of discordance among twins with polycystic ovaries, suggests a more complex pattern of inheritance than an autosomal dominant pattern of inheritance. Similarly, although some cytogenetic studies suggested that **PCOS** may de associated with a large deletion of chromosome 11(**Meyer et al., 2000**) and X chromosome aneuploidies, (**Peppard et al., 2001)** large cytogenetic studies were failed to reveal any karyotypic abnormalities (**Stenchever et al., 1986).**

Studies on candidate genes have focused on those involved in carbohydrate metabolism, genes involved in gonadotrophin action and the major histocompatibility region. These candidate genes include **CYP11A, CYP17, CYP21**, androgen receptor, SHBG gene, insulin receptor gene, insulin gene,

insulin receptor substrate gene, capain 10, FSH β-subunit gene, dopamine receptor gene and follistatin (**Legro and Strauss, 2003**).

Although there is no overwhelming evidence supporting linkage from many of these studies, the strongest evidence can be found in support of the region near the insulin receptor gene as it has been found in two different studies, where an association was demonstrated between a marker that is located 2 mega bases centromeric from the insulin receptor (**Urbanek, 1999; Tucci, 2001**).

A unique molecular phenotype of skeletal muscle and ovarian theca cells from women with **PCOS** also provides some evidence in support of a genetic etiology. In one study, increased insulin receptor serine phosphorylation of skeletal muscle was found in 50% of a sample of a women with **PCOS** (**Dunaif et al., 1995**).Although it could be argued that epigenetic alterations could have occurred during the in vitro culture of these cells, the result from microarray analysis of ovarian theca cells and skeletal muscle culture from women phenotype **(Wood et al., 2003**), large and confirmatory studies are however, awaited. An overall appraisal of the evidence in support of a genetic basis for PCOS would, therefore, suggest that although there is some evidence that genetics underpins **PCOS**, the evidence is not overwhelming, and several genes or an interaction between several genes and environmental factors may be involved (**Sharma et al., 2005**).

Insulin resistance

It is now clearly established that insulin resistance is present in obese and non-obese women with **PCOS**; however, the exact mechanisms of insulin resistance in **PCOS** remain elusive. Recent studies on mechanisms of insulin resistance in **PCOS** have focused on polymorphism in genes regulating carbohydrate homeostasis. However, none of these genes have been consistently shown to be related with **PCOS** (**Sharma et al., 2005).**