REVIEW ARTICLE

Hyperhomocysteinemia, the Player behind the Curtain: A new insight into Polycystic Ovary Syndrome

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Abstract

Polycystic ovary syndrome (PCOS), the commonest form of dysovulatoy infertility is a major form of heterogeneous endocrinopathy comprised of broad spectra of ovarian disorders including, anovulation, hyperandrogenism with trademark features of metabolic syndrome (MS) like insulin resistance, obesity and dyslipidemia. Hyperhomocysteinemia, a close associate of MS and an increasingly frequent finding among PCOS women in India, poses a possible threat to these individuals with recurrent miscarriages. Micro-thrombi formation in hyperhomocysteinemic PCOS women cues toward the development of a "thrombophilic syndrome" in these women with its possible culmination in coronary artery disease later in life. However, pathophysiology of hyperhomocysteinemia in the context of PCOS and its far-reaching consequences remains unexplored.

Keywords: hyperhomocysteinemia, metabolic syndrome, polycystic ovary syndrome

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Introduction

An ever-increasing world population with its plethora of negative connotations cannot dissuade an infertile couple their desire or right to a child of their own. Polycystic ovary syndrome (PCOS) is a common root of dysovulatory infertility afflicting reproductive aged women and a frequent reason for menstrual disorders and hyperandrogenism,1 both negatively impacting young women psychologically well as financial grounds.[2] Better understanding of this ailment amongst the general population and clinicians has taken place in recent years, with the knowledge that women diagnosed with PCOS are susceptible to metabolic syndrome (MS) and its related comorbidities.[1]. PCOS is a heterogeneous syndrome and defined multiple times by doctors with expertise in gynaecology endocrinology and psychiatry. PCOS continues to remain a largely unexplored illness and provides much scope for basic research scientists and doctors alike to attempt to illuminate its genesis and differentiate prime physiological, biochemical and pathological changes from other lifestyle induced influences.

Definition and Diagnostic Criteria

Ever since the term PCOS was coined back in 1935 by Stein and Leventhal,[3] it has undergone many revisions to enable clinicians and researchers alike to understand the basis of its diagnosis. The European Society for Human Reproduction and Embryology and the American Society of Reproductive Medicine PCOS Consensus Workshop Group[4] replaced the National Institutes of Health 1990,[5] preliminary consensus definition in 2003. More recently, the 2006 Androgen Excess Society Task Force on the Phenotype of PCOS tapered the definition to eliminate women without androgen excess.[6] In summary, women with the diagnosis of PCOS should possess at least two of the following three key features; chronic anovulation, hyperandrogenism, and/or polycystic ovaries on ultrasonography as depicted in Box 1.

In current practice, women with PCOS frequently screened for insulin resistance (IR), hypertension and abnormal cholesterol-triglyceride with other coronary prone behaviour; although the effect of individual risk parameters is unknown.[7] However, a new factor in the etiology has been noted amongst the South East Asian population; elevation in serum homocysteine levels.[8,9] Homocysteine (Hcy), a sulphur-containing amino acid primarily adjudged as an atherosclerotic marker.[10] Nevertheless, the frequent occurrence of hyperhomocysteinemia (HHcy) in PCOS generated the recent surge of interest in search for a fresh entity in the metabolic horizon of PCOS. The prevalence of PCOS varies from 5-10% depending on ethnicity and other environmental factors.[11] Whereas, among the western population, Mexican American women might have the highest prevalence of PCOS (19%),[12] incidence rate in India might range from 2.2% to 56%.[9,13,14] This alarming shift

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Box 1: Comparison of different diagnostic features of PCOS over the years

Year/Criteria	*Dysovulation*	*Hyperandrogenism*	*Polycystic Ovaries*	*Exclusion Criteria*
1990/NIH (4)	Less than six menstrual cycles per year	Clinical/biochemical	No	Other known disorders
2003/Rotterdam ESHRE/ARSM(5)	Oligo ovulation	Clinical (hirsutism) / biochemical (increased free levels of testosterone)	Yes	Disorders with similar clinical presentation
2006/AES (6)	Yes/No	Clinical (hirsutism) / biochemical (increased free levels of testosterone)	Yes/No	Other androgen excess and related disorders

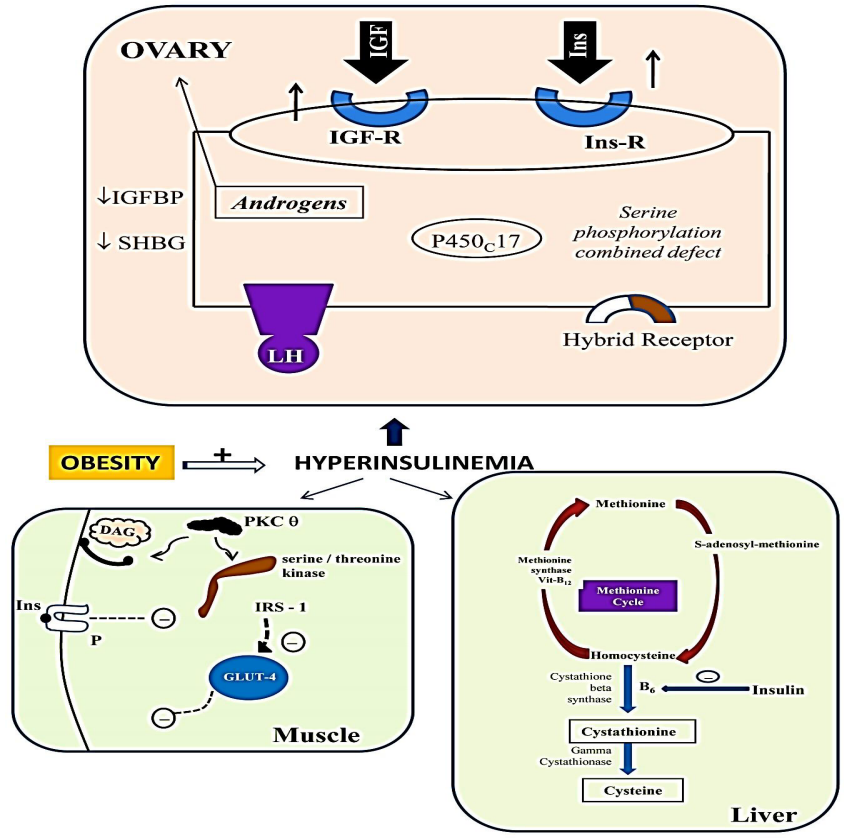

Figure 1: Hyperinsulinemia act as the central player in the crossroad of steroidogenic and homocysteine metabolism to drive the androgen production in Polycystic Ovary Syndrome.

coincides with the strikingly high prevalence rate of HHcy in Indian subcontinent and amongst the Indian immigrant population in UK as well.[15] This probably cues towards the development of a novel risk factor in PCOS from an Indian perspective.

Pathophysiological Analysis

Insight into the pathogenesis of PCOS has increased substantially in the last decade. However, it is believed that IR, master of the metabolic orchestra in PCOS, results in elevated insulin levels, which in turn initiates a chain of mechanisms such as inhibition of sex hormone binding globulin (SHBG) production within the liver thereby raising levels of free testosterone. Amplification of androgen levels in the theca cells has been observed because of the inhibition of insulin growth factor-1 binding protein (IGFBP-1). Metabolic factors aside, genetic (serine phosphorylation or pertaining to the area around the insulin receptor gene at chromosome 19p13.3) and environmental changes like over eating and sedentary lifestyles also play cogent roles.[15]

In recent years an increased incidence of CVD has been documented in PCOS.[16] Incidentally, a considerable population of PCOS women does also suffer from HHcy, a close associate of IR and an independent risk factor of MS.[14] HHcy has been adjudged detrimental to oocyte quality and function leading to pregnancy wastage.[17] A recent paper documents that HHcy results in coagulation dysfunction and produce micro thrombi in uterine vessels. This perturbs embryo implantation cueing to early pregnancy loss in women with PCOS.[9] In spite of numerous alliances between PCOS, IR and HHcy, (Figure 1) the cause and effect relationships between these three entities still remain

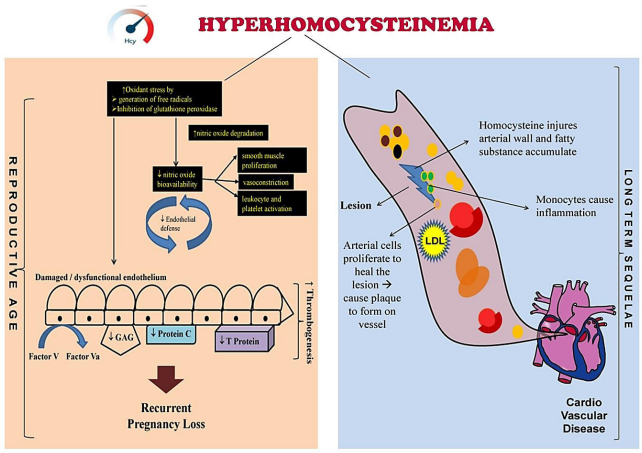

Figure 2: The more the worse: hyperhomocysteinemia draws the fate in an individual with polycystic ovary syndrome in both short as well as in long term featuring pregnancy loss and metabolic facets.

elusive and it remains yet to be resolved if HHcy co-exists independently, an after-effects of the disease or it has any contributory role in the pathogenesis of the disease. With current advances in diagnostic approaches HHcy can be considered as the tip of the iceberg directing PCOS into metabolic and thrombophilic directions, thereby helping us to unravel and understand its far-reaching consequences.

Long-term Health Consequences of PCOS

Women diagnosed with PCOS face lifelong health ailments as this syndrome carries lasting implications for a woman's overall health and general well-being.[18] These consequences can be broadly classified into metabolic and thrombophilic directions. Several women diagnosed with PCOS, exhibit signs and symptoms of metabolic syndrome which include obesity, IR and dyslipidemia.[19] These risk factors translate into the long-term risk/s of PCOS and eventual build-out of type 2 diabetes mellitus (T2DM) and CVD or pregnancy loss following the thrombophilic avenue. HHcy, by virtue of its dual switch mechanism controls IR, thereby acting as a fulcrum to direct the long-term sequences of the syndrome into either of the avenues. Hence, the extent of HHcy is not only as a risk factor in PCOS but also as forbearer of various constant complaints. However, the question as to whether HHcy *per se* carries increased risk or an epiphenomenon linked to the obesity and/or IR causing metabolic or thrombophilic derangements, remains unanswered.

Risk Factors Distinguishing the Fate of the Individual in PCOS

Glucose Intolerance, Type 2 Diabetes and Insulin Resistance

In general, an approximate 3 to 7 fold greater risk of T2DM and impaired glucose tolerance is observed in women with PCOS compared with the control counterpart/s.[20] Hyperinsulinemia, a consistent finding in women with PCOS in their reproductive years is a contributory factor in PCOS related pregnancy loss. Study by Jakubowicz *et al* 2002[21] documented lower serum glycodelin and serum IGFBP -1 levels during week 3 to 8, and week 9 to 11 respectively in women with PCOS having early pregnancy loss. The potential links between IR and repetitive pregnancy loss in PCOS in recent studies showed a significant reduction of first-trimester loss in women treated with insulin-sensitizing drugs.[22] However, additional research is necessary to determine the prevalence as well as the underlying mechanism of RPL in PCOS. However, IR continues to be considered the main link between diabetes and CVD in PCOS patients in later years of life. The direct atherogenic action and aberration in the lipoprotein profile brought by IR in PCOS propels towards a dysbalance in the lipid profile through dyslipidemia, which finally culminates at CVD to complete the full circle of the health consequences in the life of a PCO individual. (Figure 2)

Obesity

The association between obesity, hyperandrogenemia and insulin resistance is well recognized. Presence of an augmented waist/hip ratio with a company of brown adipose tissue in obese women with PCOS increases the hassle of weight gain despite dietary regimens and exercise.[23] Obese-PCOS phenotype have a strong predisposition to recurrent miscarriage, perhaps provoking thrombosis via several mechanisms, including increased activity of the coagulation cascade and decreased activity of the fibrinolytic cascade. However, it remains unclear if obesity itself or obesity-associated comorbidity, such as IR, is responsible for this phenomenon. Obesity-induced IR triggers dyslipidemia in PCOS, ensuing long-term health consequences (CVD) later in life.[24]

To sketch a unifying mechanism linking obesity, IR, and the comorbidities of the syndrome, it seems that a defect in insulin release by the β-cell could be crucial.[25] Android obesity and centripetal fat distribution renders PCOS individuals more insulin resistant.[26] Microarray of omental adipose tissue biopsies have revealed abnormalities in several gene cascades/s of insulin signalling pathway in women with PCOS specifically with the over-expression of phosphotidyl inositol 3 kinase receptor-1.[27] The increased protein kinase A–hormone sensitive lipase complex activity in visceral fat cells could cause an early lipolytic defect in young women with PCOS.[28] However, the presence of two life-changing ailments, CVD and RPL and the yet-to-be-determined unique etiology of their separate pathogenesis cannot be ignored.

Hyperhomocysteinemia: Iantern Guiding PCOS Individual Towards her Destined fate

HHcy, an independent and graded risk factor for the development of CVD,[29] recently gained importance amongst gynaecologists as higher cardiovascular morbidity is a frequent phenomenon observed in PCOS patients.[30] Hcy is formed during the breakdown of an amino acid methionine,

which may undergo remethylation to methionine, or trans-sulphuration into cyatathione and cysteine.[31] Interestingly, insulin inhibits Hcy catabolism at trans-sulphuration by the inhibition of cystathione-β-synthase (CBS) activity, as documented by i*n vitro* studies.[32] Hence, under condition of IR, Hcy concentrations would be increased and this concept stands true with the increased Hcy concentrations frequently observed in insulin-resistant condition/s, especially, in sub-continental[9] and Sri Lankan population (52 to 84%)[8,9,33] in contrast to the west (5-7%).[34] However, Hcy levels is subjective to other factor/s than insulin. This emergence of HHcy has led us to search for the hidden factor in etiopathology in Indian population. Hepatic folate, methyl groups and catabolic products of the amino acid itself provide a link between several intermediary pathways.[35] They have a wide range of functions, including epigenetic regulation of gene expression and maintenance of redox status.[36] Enzymatic defects caused by genetic mutations (e.g., C677T, A1298C) in Hcy metabolism induce a significant increase in serum Hcy concentrations.[37] The commonest cause of HHcy seems to be due to a reduced efficiency of methylenetetrahydrofolate reductase (MTHFR), an enzyme involved in folate-dependent remethylation.[38] Surprisingly, up to one-third of Indians have a genetic defect, which predisposes to decreased activity of MTHFR.[39] Other than MTHFR, the incidences of mutation in the South-Asian perspective include A2756G polymorphism in methionine synthatase and T833C/ 844ins68 and G919A polymorphisms in CBS gene.[40]

On the whole, one or more defects in the one-carbon pool along with the inhibition of insulin create the state of HHcy thereby striking the chord for the initiation of metabolic morbidities.

PCOS and MS both share a common locus- IR that bestows increased risk for T2DM, the prime medical outcome of both syndromes. Impaired insulin sensitivity through its transitional corridors paves the way to CVD. A study on rodents in 2000 has shown that high fat and sucrose fed diet-induced IR decreased CBS and compensatorily increased MTHFR activities, followed by HHcy.[41] Bellia *et al*. in 2007[42] reported that a collective operation of HHcy and MS can substantially increase CVD risk than HHcy and/or MS alone. (OR 13.11, 95% CI 5.27–32.06) while Hajer[43] showed contribution of HHcy is greater when it comes to its deleterious consequences. In addition, an increased plasminogen activator inhibitor type-1 in women with PCOS,[44] a potential inhibitor of fibrinolysis and inducer of coronary artery calcification[45] paves the path for the progression towards CVD. Although clinician/s routinely prescribe folate and vit. B12 to control HHcy;[46] the role of the duo in reducing CVD risk is is debated.[47] It was observed that although a continuous vitamin supplementation reduces plasma Hcy level, it has a minimum effect on the risk of death resulting from cardiovascular disease.[29] These findings put forward the underlying presence of other metabolic or genetic impairments on Hcy metabolism to exert its negative effect on cardiovascular risk profile.

However, one mechanism or two is believed to contribute to atherosclerosis via HHcy. HHcy by producing increased free radicals and creating a state of oxidative stress convert LDL-cholesterol (LDLc) to oxidized LDLc (OxLDLc) which chiefly operates in the inflammatory process turning out to atherosclerosis.[48] OxLDLc mediates the release of vascular cell adhesion molecule and monocyte chemo-attractant protein, which in turn causes monocyte adhesion and penetration, respectively. The monocyte to macrophage conversion requires OxLDLc which ultimately forms foam cells and gets deposited below the endothelium to form a fatty streak, the first lesion in atherosclerosis.[34] This action synergizes with the inactivation of nitric oxide, a potent vasodilator by reactive oxygen species.[49] The summarizing stroke result to endothelial dysfunction, contributing to atherosclerosis.

Conclusion

There is little doubt that women with PCOS cluster risk factor for diabetes, CVD and recurrent miscarriage.[50] HHcy is a proven risk factor for CVD-inducing arterial and venous thrombosis. According to the present dictum, increased Hcy levels in serum can be lowered by the administration of folic acid, vitamin B6, and vitamin B12 or a combination of the three. However, controlling HHcy does not merely reduce the risk of thrombosis and resulting atherosclerosis. Since the report/s on the topic is not univocal, it appears prudent to lower one's Hcy levels through supplementation.

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