

## Association between Hypertension and Lipid Profile with Homocysteine in PCOS

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### Abstract:

**Background:** Homocysteine is one of the most destructive compounds found in the human body. Among women with PCOS, hyperhomocysteinemia is found to have a role in infertility, miscarriages, pre-eclampsia, placental abruption and intra uterine death due to placental insufficiency **Methods:** This was a hospital based cross sectional study on 50 PCOS women at SAT hospital, Govt Medical College, Thiruvananthapuram. Blood pressure of the study population was recorded and biochemical parameters like fasting plasma Homocysteine, Fasting lipid profile were estimated.

**Results:** Hyperhomocysteinemia was prevalent among 52% of the study population. Mean Homocysteine value obtained in the study was  $9.5 \pm 3.6$ . Among the 50 women studied hypertension was observed among 24 women; 43(86%) were found to have elevated low density lipoproteins (LDL) levels and 46% of women had low HDL levels. But there was no association between blood pressure and lipid profile with hyperhomocysteinemia.

**Conclusion:** Homocysteine, via its thiolactone derivative play a very decisive role in the pathogenesis of neural tube defects, miscarriages, preeclampsia, gestational diabetes, intra uterine deaths among PCOS women. Early detection of Hyperhomocysteinemia along with life style modifications can prevent further complications in PCOS.

**Keywords -** Homocysteine, Hyperhomocysteinemia, PCOS

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### I. Introduction

Polycystic ovarian syndrome (PCOS) is the leading cause of menstrual dysfunction, infertility, recurrent abortions and is associated with insulin resistance and hyperandrogenism, which results in long term health consequences like coronary artery disease, diabetes mellitus, systemic hypertension and endometrial carcinoma. Hyperhomocysteinemia in PCOS amplifies insulin resistance and hyperandrogenism in PCOS via its derivative Homocysteine thiolactone. Nutritional deficiencies of vitamin B-6, vitamin B-12 and folic acid and usage of Glyciphage in PCOS lead to Hyperhomocysteinemia (Hcy).<sup>1-7</sup>

The oxidant stress of Hcy trigger endothelial dysfunction and injury (Blundell et al., Loscalzo)<sup>8,9</sup> resulting in reduced vasodilatory capacity, activation of circulating white blood cells and platelets, activation of prothrombotic mechanisms, inhibition of fibrinolytic mechanisms and stimulation of vascular smooth muscle cell proliferation. This can lead to pre-eclampsia or cause placental insufficiency resulting in intra uterine growth restriction or even death of the fetus during pregnancy.<sup>10</sup>

Several population-based studies have linked plasma Homocysteine levels to blood pressure, especially systolic pressure. Mechanisms that could explain the relationship between Homocysteine and blood pressure include increased arterial stiffness, endothelial dysfunction with decreased availability of nitric oxide, low folate status, and insulin resistance.<sup>10</sup> Cross-sectional studies by Conway et al. in 1992, Talbott et al. found a positive correlation between Homocysteine (Hcy) and blood pressure.<sup>11</sup> In a community-based case-control study conducted in China, among 127 essential hypertensive patients and 170 normal subjects, aged 35 to 75, to examine the relationship between abnormal Hcy metabolism and essential hypertension, no correlation was obtained between Hcy and hypertension.<sup>12</sup>

Women with polycystic ovary syndrome (PCOS) appear at increased cardiovascular risk due to dyslipidemia, characterized by increased plasma triglyceride, reduced high density lipoprotein (HDL) cholesterol levels and raised LDL levels.<sup>13</sup> Metabolic syndrome is a mutual soil for Hcy as well as low HDL, resulting in a high risk for coronary diseases. A positive association between Hcy and lipids has been also documented in studies on individuals with mild Hcy. In the Hordaland study, a higher consumption of

saturated fatty acids was positively linked with higher plasma Hcy.<sup>14</sup> A study conducted by Xiao et al on found a positive correlation between Hcy and LDL, TC, and TG levels and negative correlation with serum HDL levels.<sup>15</sup> A study to assess Homocysteine (Hcy), vitamin B12 and folic acid (FA) concentrations in 137 resident Indian women and to study their correlation with traditional risk factors for coronary artery disease, by SN Pandey et al., couldn't find any correlation between Homocysteine and lipid profile.<sup>16</sup>

Hhcy induce hepatic cholesterol biosynthesis and lipid accumulation, via activation of various transcription factors. Liao et al and Mikael et al. reported that Homocysteine reduces the concentration of HDL cholesterol in plasma, by inhibiting the hepatic synthesis of Apo A-I (the main HDL apolipoprotein) or its expression by lowering PPAR $\alpha$  (Peroxisome Proliferator- Activated Receptor alpha ). Low PPAR $\alpha$  is also responsible for increased expression of cholesterol 7 $\alpha$ -hydroxylase (CYP7A1) and enhances cholesterol absorption in the intestine. SAH- hydrolase, the enzyme that converts Hcy to SAH (S-Adenosyl Homocysteine) play a major role in Hcy metabolism and cellular lipid homeostasis. SAH accumulation results in deregulated lipid metabolism, leading to an imbalance in phospholipid and triglyceride synthesis, with probable implications for mammalian lipid-associated disorders.<sup>17,18</sup>

## II. Methods

The study was a hospital based cross sectional study done on 50 PCOS patients at Sree Avittam Thirunal Hospital (SAT), Government Medical College, Thiruvananthapuram. The study was conducted for a period of one year.

### 2.1 Inclusion criteria

The Rotterdam's criteria were used to identify the PCOS patients. Females within 15-35 years identified with any two of the criteria shown below<sup>19</sup>

- a) Oligo-ovulation or anovulation manifested as oligomenorrhea or amenorrhea.
- b) Hyperandrogenism (clinical evidence of androgen excess-hirsutism, acne).
- c) Polycystic ovaries (as defined on ultrasonography).

### 1.2 Exclusion Criteria

- a) Patients who were on folate antagonists/ folic acid
- b) Patients who had renal diseases.
- c) Patients who were diabetic/hypertensive.
- d) Patients who were smokers.

The study was conducted after getting clearance from human ethical committee and review board of the institution. A written informed consent was obtained from all persons included in the study. A detailed history taking and thorough clinical examination of patients were done. History of folic acid intake was enquired blood pressure of the study group were recorded and hypertension was excluded. A sonography analysis was performed for the confirmation of PCOS. Fasting blood sample was collected from patients for estimation of Homocysteine levels and fasting lipid profile.

Blood pressure was recorded using sphygmomanometer. PCOS women have a high predisposition to hypertension. Hence their BP level should be <130/80 mm Hg.<sup>20</sup> In the current study hypertension is defined, not according to the standard guidelines and any BP value >130/80 mm Hg is considered as hypertension.

Hyperhomocysteinemia is defined as an increased fasting plasma Homocysteine levels. Reliable reference intervals for concentrations of Homocysteine in plasma have not yet been established. The normal range of plasma Hcy levels usually accepted are 5-12 $\mu$ mol/L.<sup>21</sup> but still it is a matter of debate. The Nutrition Committee of the American Heart Association proposed <10  $\mu$ mol/L as a reasonable fasting Hcy value, to avoid all complications of high Homocysteine levels.<sup>22</sup> Accordingly in the current study, value  $\geq 10\mu$ mol/L is considered as hyperhomocysteinemia.<sup>23</sup>

The Adult Treatment Panel III (ATP III) of the National Cholesterol Education Program<sup>24</sup> issued an evidence-based set of guidelines on cholesterol management in 2001. According to these guidelines, in the current study, desirable lipid profile and abnormal plasma lipid values are defined as follows –

Lipid subfractions	Desirable Levels (normal)	Abnormal plasma levels
Total Cholesterol	$\leq 200$ mg/dl	$> 200$ mg/dl
Triglycerides	$\leq 150$ mg/dl	$> 150$ mg/dl
LDL Cholesterol	$< 100$ mg/dl	$\geq 100$ mg/dl
HDL Cholesterol	$> 35$ mg/dl	$\leq 35$ mg/dl
VLDL Cholesterol	$\leq 35$ mg/dl	$> 35$ mg/dl

**Statistical analysis**

The data were entered into a personal computer using the package Microsoft excel. For analysis SPSS (Statistical Package for Social Sciences) Window version 16 was used. Continuous variables were expressed as mean ±standard deviation and qualitative data was expressed as percentage. Correlations between variables were done using Pearson correlation test. A ‘p’ value <0.05 was considered statistically significant.

**III. Results**

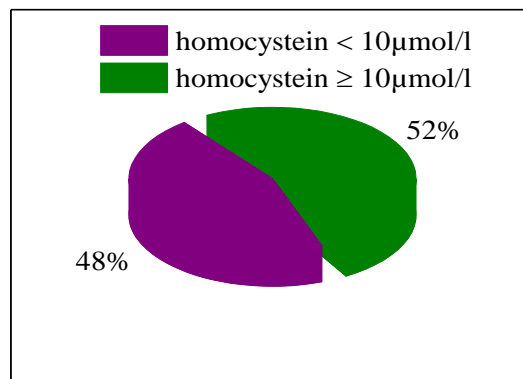
**Table: 1** Characteristics of the study group

Study Variables	N	Mean	SD
Age (years)	50	26.5	4.9
Homocysteine (µmol/l)	50	9.5	3.6
Tot Cholesterol (mg/dl)	50	190.1	30.1
LDL (mg/dl)	50	132.8	27.8
HDL( mg/dl)	50	36.6	8.3
TG (mg/dl)	50	126.3	49.2
VLDL( mg/dl)	50	23.7	8.9

**Table 2:** Distribution of patients based on Homocysteine levels

Homocysteine	Frequency	Percentage
≥10 µmol/l	26	52.0
<10µmol/l	24	48.0
Total	50	100.0

The proportion of hyperhomocysteinemia (defined as ≥10µmol/l) was 52% among the study population. Mean Homocysteine in the study was 9.5±3.6 µmol/l.



**Fig 1-**Pie diagram showing distribution of patients with Hyperhomocysteinemia

**Table 3** Distribution of patients based on their blood pressure status

Hypertension (mmHg)	Frequency	Percentage
Present ( BP >130/80)	24	48.0
Absent (BP ≤130/80)	26	52.0
Total	50	100.0

The association between PCOS and hypertension has shown conflicting results. Similarly in our study also, hypertension was observed among 24 women, among the 50 women studied.

**Table 4** Distribution of patients based on their lipid profile status

Subtractions of lipid profile	Cutoff values	Frequency	Percentage
Tot Chol	>200 mg/dl	18	36.0
	≤200 mg/dl	32	64.0
LDL	≥100mg/dl	43	86
	<100mg/dl	7	14
HDL	≤35 mg/dl	23	46.0
	>35 mg/dl	27	54.0
TG	>150 mg/dl	11	22.0

	≤150 mg/dl	39	78.0
VLDL	>35 mg/dl	5	10.0
	≤35 mg/dl	45	90.0

Dyslipidemia is common in women with PCOS, although the extent and type of dyslipidemia have been variable. Among the 50 patients studied, 43(86%) were found to have elevated low density lipoproteins (LDL) levels and 46% of women had low HDL levels. High LDL level together with a low HDL are vital risk factors for atherosclerosis

**Table 5** Association between Homocysteine and hypertension

Hypertension	Homocysteine			
	≥10 μmol/l		<10 μmol/l	
	N	%	N	%
Present	14	58.3	10	41.7
Absent	12	46.2	14	53.8
Total	26	52.0	24	48.0

$\chi^2 = 0.7$                        $p = 0.389$

Among the study group, out of the 24 hypertensive women, 14 (58.3%) had hyperhomocysteinemia. Also among the 26 non hypertensive women, 12 (46.2%) had hyperhomocysteinemia. There was no association between hypertension and homocysteine (p=0.389).

**Table 6** Pearson Correlation between Homocysteine levels and other factors

Other parameters in the study	Pearson Correlation (r)	p value
Tot Chol mg/dl	-.026	.855
LDL mg/dl	.029	.842
HDL mg/dl	.145	.314
TG mg/dl	.032	.824
VLDL mg/dl	-.130	.368

In this study, no correlation was observed between Homocysteine and lipid profile

#### IV. Discussion

In our study 52% of PCOS patients had hyperhomocysteinemia. Insulin resistance, hyperinsulinemia and use of Glyciphage ( Metformin) to improve insulin sensitivity cause Hhcy in PCOS.<sup>25</sup> Insulin and Hcy have the ability to induce each other. Insulin levels have been integrated to increased Hcy levels, as insulin inhibits the 2 key enzymes for Hcy metabolism- MTHFR ( Methylene Tetra Hydro Folate Reductase ) and hepatic CBS (Cystathionine Beta Synthase) . Hhcy through homocysteine thiolactone, inhibits the tyrosine kinase activity of insulin receptor, affects insulin signaling and induces insulin resistance, resulting in hyperinsulinemia, leading to the accumulation of Hcy in plasma Thus, insulin resistance and Hhcy create a harmful feedback loop, each stimulating the development and propagation of the other and upsurge all complications of PCOS.<sup>26-28</sup>

Homocysteine, due to the presence of –SH group or sulfhydryl group undergoes auto oxidation and produce reactive oxygen species and aggravates the oxidative stress in PCOS which can badly influence female reproduction by affecting oocyte quality or oocyte function, oocyte penetration and fertilization, causing delayed conception, improper implantation or loss of an implanted embryo.<sup>29</sup>In addition, oxidative stress has an impact on insulin signalling leading to insulin resistance, endothelial dysfunction and atherosclerosis. Hhcy induced endothelial dysfunction and injury lead to pre eclampsia or cause placental insufficiency resulting in intra uterine growth restriction or even death of the fetus during pregnancy.<sup>30,31</sup>

In our study 48% of the study population had hypertension, but no significant association was obtained between Hcy and hypertension. (p=0.389)[Table 5]. This was in accordance with the study conducted by Zhans et al. in China, among 127 essential hypertensive patients and 170 normal subjects, to examine the relationship between abnormal Homocysteine metabolism and essential hypertension, but no correlation was obtained between the two.

The foremost reason for this observation in our study would be our small sample size. Also in the study group plasma Hcy levels were not that significantly elevated for inducing vasculopathy that can cause hypertension. The young age of our study group may be another reason. An additional explanation would be that the high estrogen in PCOS might have caused a reduction in the plasma Hcy levels, as evidenced by many previous studies that have proved the Hcy lowering effect of estrogen.

Hcy lowering effect of estrogen was observed in the studies by Andersson A et al. and Wouters MG et al. They observed that Hcy levels were found to be higher in post-menopausal women, when compared to premenopausal women, suggesting a role for estrogen in decreasing Hcy levels.<sup>32,33</sup> Another prospective study

by Ozgur Baris Gul et al. also proved this observation.<sup>34</sup> The 3<sup>rd</sup> National Health and Nutrition Examination Survey in their publication has proposed that, increased Estrogen status is associated with a decreased mean serum total Hcy concentration, autonomously of nutritional status and muscle mass, and that estrogen may explain the male–female difference in total Hcy concentration.”<sup>35</sup>

Estrogen increases Cystathionine-β-Synthase activity and direct Hcy metabolism towards cysteine and glutathione formation. Estrogen also up-regulates NOS (Nitric Oxide Synthase) activity and increases concentration of nitric oxide. Estrogen enhances the activity of glutathione peroxidase, the enzyme that catalyzes the scavenging of oxygen free radicals by glutathione. Estrogen also limits the ONOO, (peroxynitrite) formation, by favoring the production of GSNO (S-Nitrosoglutathione), which is a redox form of nitric oxide and convey specific cyto-protective effects.<sup>32-35</sup>

Dyslipidemia is common in women with PCOS, although the degree and type of dyslipidemia have been variable. Elevated Low density lipoprotein (LDL) levels have been reported in several studies of women with PCOS, a finding not usually noted in insulin-resistant states, suggesting a varied origin for dyslipidemia in these women.<sup>36</sup> Similarly in our study, among the 50 cases studied, 43 women (86%) were found to have high LDL levels [Table 4]. But in our study no significant association was found between Hcy and any component of lipid profile ( $p > 0.05$ ) [Table 6].

Hcy in conjunction with high LDL amplifies the likelihood for coronary artery disease. Strong evidences suggest that excess of plasma Hcy disturb lipid metabolism by promoting the oxidation of LDL particle and its aggregation, thus favouring atherogenesis. Aggregates formed by the combination of Hcy thiolactone with LDL are taken up by intimal macrophages and incorporated into atheromatous plaques (Naruszewicz et al., 1994). This also promotes proliferation and fibrosis of smooth muscles. Highly reactive oxygen species are generated by Hcy thiolactone -LDL complexes, which cause several changes in the intima of the blood vessels and thereby endothelial dysfunction, resulting in development of atherosclerotic plaque.<sup>37,38</sup>

In the current study, 46% of women had low HDL levels [Table 4]. The high density lipoprotein (HDL) particle is known to prevent the formation of ox-LDL (oxidized LDL), through the HDL-associated enzyme Paraoxonase (PON). It is a multifunctional antioxidant enzyme that not only can destroy ox-LDL but also can detoxify homocysteine thiolactone (Jakubowski H, 1997). Therefore, a high LDL in association with low HDL levels amplifies the possibility of CAD, as evidenced by previous studies.<sup>38</sup>

The possible reason for the lack of association between Hcy and lipids in the present study can be the insufficient sample size. To predict correct association between them, more studies on large samples are needed. However increase in LDL levels among the study group demand strict dietary restrictions and life style modifications in these women.

The intense treatment of hyperhomocysteinemia in women with polycystic ovary syndrome might improve reproductive outcome and promote protection from the possible cardiovascular risks. This may be achieved by the early screening, lifestyle education, and vitamin B6, B12 and folic acid supplementation. Various steps to lower Hcy levels include consumption of well balanced diet with plenty of fish, garlic, green leafy vegetables and other vegetable protein. Avoidance of fatty meat and reduction in tea, coffee and excess salt consumption is recommended.

### **Limitations**

The foremost limitation of our study was that we estimated the Homocysteine levels and other parameters among a small group of PCOS women.

### **V. Conclusions**

Our study recommends screening of all PCOS women for an elevated homocysteine level, especially for those who are obese and with a family history of Type 2 DM and CAD. Since the estimation of serum Homocysteine level by Chemiluminescence assay is very expensive, the affordability can pose problems.. Hence, a more cost effective and sensitive method for estimation of plasma homocysteine is to be formulated and the government should formulate policies to make estimation of this risk factor affordable for our general population, at subsidized rates.

Further studies are required to find out the reference value for Homocysteine in PCOS of our native population. For this, the B vitamin status of our population needs to be explored, since it is the major determinant of serum homocysteine levels. Also more studies on MTHFR gene polymorphism and its distribution are needed, so as to elucidate the reasons for the high prevalence of hyperhomocysteinemia among Indians.

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