VOLUME-8, ISSUE-2, FEBRUARY-2019 • PRINT ISSN No 2277 - 8160

Original Research Paper

Biochemistry

# EVALUATION OF HOMOCYSTEINE, C-REACTIVE PROTEIN AND LIPID PROFILE AS MARKERS OF CARDIO VASCULAR RISK IN WOMEN WITH POLYCYSTIC OVARIAN SYNDROME [PCOS]

**Dr. P. R. Vanaja** M.D., Assistant Professor of Biochemistry, Kurnool Medical College, Kurnool.

**Dr. Shruthi Mohanty*** M.D., Professor & H.O.D., Kamineni Institute of Medical Sciences, Hyderabad *Corresponding Author

## ABSTRACT

**Objective:** To determine Cardiovascular risk in patients with polycystic ovary syndrome (PCOS) and its relation to C-reactive protein (CRP) and homocysteine (Hcy) levels.

**Design:** Cross sectional study.

**Setting:** Kamineni Institute of medical sciences and hospital from 2012- 2014

**Patient(s):** Fifty women with PCOS and fifty healthy women were included this study.

**Intervention(s):** Cardiovascular risk was evaluated by measuring serum levels of CRP and Hcy and lipid profile.

**Results:** There was an elevation of TC/HDL in 44% cases, with (P – 0.095) , elevation of LDL/HDL in 24%, cases with (P – 0.079) and lower levels of serum HDL in 58% cases, with (P – 0.001) . There was an elevation of serum Homocysteine levels in 58% cases, with (P – 0.001) . There was an elevation of serum CRP levels in 58% cases, with (P –0.001). In correlative studies Homocysteine correlates positively with VLDL(P-0.293) and CRP positively correlates with TC(0.037) and VLDL-C ( P-0.157). .

**Conclusion(s):** In conclusion the findings of the study show significantly elevated levels of both lipid profile and homocysteine as cardiovascular risk markers in PCOS women. The various parameters like Serum TC, TG, LDL, VLDL, TC/HDL ratio, LDL/HDL ratio and TG/HDL ratio, homocysteine and CRP levels were elevated in the study group and also there was a reduction in the HDL levels in the study group. At the same time elevated inflammatory risk markers CRP and homocysteine can cause endothelial dysfunction and thus increase the risk of early onset cardiovascular risk in these young women.

**KEYWORDS** : Cardiovascular risk, Lipid profile, CRP, Homocysteine and polycystic ovary syndrome

## Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrinological disorder amongst reproductive age women. The pathophysiology of PCOS is complex in that it causes a spectrum of manifestations (1-4). It is associated with a variety of comorbidities such as diabetes, hypertension, dyslipidemia, cardiovascular events and malignancies that manifest at a young age (4,5). Preliminary investigations suggest that serum biomarkers of cardiovascular disease such as highsensitivity C-reactive protein, homocysteine and adiponectin are abnormal in women with PCOS (6-9). Since hyperhomocysteinemia is a risk factor for cardiovascular diseases, it has been postulated that homocysteine levels are higher in PCOS patients than controls. Homocysteine is an amino acid formed by the conversion of methionine to cysteine. It is metabolized by one of two pathways: trans-sulfuration and remethylation. This process requires vitamin B as a cofactor (5-8). Normal homocysteine concentrations range between 5 and 15 µmol/L. Hyperhomocysteinemia has been classified as follows (10): mild (15 to 30 µmol/L), intermediate (30 to 100 µmol/L) and severe (>100 µmol/L). Elevations in plasma homocysteine are common and occur in five to seven percent of the general population. Hyperhomocysteinemia is an independent risk factor for atherosclerotic vascular disease, cerebrovascular events and recurrent venous thromboembolism. It can occur due to genetic defects in the enzymes involved in homocysteine pathways such as methylene tetrahydrofolate reductase (MTHFR), to deficiencies in vitamin cofactors, or additional factors which include certain chronic medical conditions and drugs, such as fibrates and nicotinic acid (11-17). Several studies have investigated homocysteine levels in PCOS patients (18-28). Most have shown that women with PCOS have elevated homocysteine levels when compared with controls (19-24). Recently, Mancini et al. in their prospective case-control study on 44 patients have shown that homocysteine levels did not differ between PCOS and control women (18). Because of the complex limitations of these studies, such as a lack of uniformity in the definition of PCOS and information on levels of other cofactors, the results vary. The purpose of this paper is to evaluate homocysteine levels in the PCOS population compared with controls.

## Materials and Methods

Fifty patients with PCOS who had no additional metabolic or cardiovascular diseases were enrolled in a prospective nonrandomized case-controlled clinical study. PCOS cases (n=50) and controls (n=50) were recruited from the Gynecology Dept at Kamineni Institute of Medical Sciences and Hospital, Nalgonda,Telangana between 2012-2014. All cases had a confirmed diagnosis of PCOS by ultrasonography. Other causes of hyperhomocysteinemia such as deficiencies in vitamins B12, B6, folic acid, and history of chronic medical conditions which included renal failure, thyroid dysfunction, liver diseases, drugs such as fibrates and nicotinic acid and smoking were previously excluded. All control women had regular menstrual cycles. Therefore, none of the control women fulfilled any of the Rotterdam diagnostic criteria for PCOS. All control women had regular menstrual cycles and normoandrogenaemia. No confounding medications (hormonal preparations, Metformin, Simvastatin and other related medications) had been taken by any of the subjects within three months of the study in both groups. A perfect match was obtained for body mass index (BMI) between PCOS cases and controls. The study was approved by the Ethics Committee of Kamineni Institute of Medical Sciences and Hospital,Nalgonda,Telangana. All subjects provided fully informed consents.

Serum homocysteine (total) levels were measured using the ELISA technique . All blood samples were taken following an overnight fast.

For comparisons of the pair-matched groups of PCOS cases and controls, we used paired-sample t-tests. All analyses were conducted with SPSS (version 12.0 for Windows; SPSS Inc., Chicago, IL, USA). P<0.05 was considered significant.

## Results

50 randomly selected PCOS women in age group between 18-45 yrs were evaluated for cardiovascular risk by estimating serum lipid profile, Homocysteine and C-reactive protein.

The mean age of women was 29 ± 5.9 years in the PCOS group and

27.3 ± 5.3 years in controls (p=0.06). The mean baseline BMI was 26.7 ± 4.2 kg/m$^2$ and 25.8 ± 4.1 kg/m$^2$ in PCOS and controls, respectively (Table 1). According to the t test there were no meaningful differences between these groups in BMI (p=0.1). The mean level of homocysteine was 18.23 ± 7.07 µmol/L in patients with PCOS and 12.10 ± 3.32 µmol/L in controls.(Table 1)

**Table 1 Demoghraphic data in patients with PCOS and controls**

| | PCOS (n=50) | Controls (n=50) |
|---|---|---|
| **Age** | 29 ± 5.9 | 27.3 ± 5.3 |
| **BMI (kg/m$^2$)** | 26.7 ± 4.2 | 25.8 ± 4.1 |
| **Homocysteine level (mean ± SD) µmol/L** | 18.23 ±7.07 | 12.10 ± 3.32 |

**Table 2 Comparison of hyperhomocysteinemia in PCOS patients and controls***

| | Hcy**(µmol/L) | P value |
|---|---|---|
| PCOS (n=50) | 18.23±7.07 | 0.001 |
| Controls (n=50) | 12.10±3.32 | |

**Homocysteine levels

Paired-sample comparisons revealed that patients with PCOS had a significantly higher risk for hyperhomocysteinemia when compared with BMI matched control women(Table 2

**Table 3: Comparison CRP, Homocysteine And Lipid Profile In Pcos And Controls**

| Analyte | PCOS Cases | Control | P value |
|---|---|---|---|
| CRP | 5.46±3.05 | 1.78±1.71 | 0.001 |
| Homocysteine | 18.23±7.07 | 12.10±3.32 | 0.001 |
| Triglycerides | 151.24±63.97 | 107.25±33.29 | 0.001 |
| Total Cholesterol(TC) | 171.66±37.14 | 148.72±26.42 | 0.002 |
| HDL Cholesterol | 36.73±3.65 | 45.15±4.16 | 0.001 |
| LDL Cholesterol | 105.75±37.90 | 80.57±29.61 | 0.001 |
| TC/HDL | 10.17±4.67 | 6.35±3.29 | 0.095 |
| LDL/HDL | 2.87±0.85 | 1.78±0.80 | 0.079 |

50 randomly selected PCOS women in age group between 18-45 yrs were evaluated for cardiovascular risk by estimating serum lipid profile, Homocysteine and C-reactive protein, were compared with 50 age matched controls.

The levels of lipid parameters TC, LDL, TG, VLDL and HDL were measured along with serum homocysteine and CRP levels. There was an elevation of TC, TG, LDL, VLDL,TC/HDL, LDL/HDL in cases when compared to controls. There was lower levels of serum HDL in cases when compared to controls . There was an elevation of serum Homocysteine levels and serum CRP in cases when compared to controls .The Atherogenic index of plasma(AIP) in controls it was 0.03 where as in cases it is 0.30 .

The elevated lipid parameters and elevated homocysteine and CRP contributes to the cardiovascular risk in atherosclerosis. Based on early recognition of PCOS, efforts may be done to limit or forestall the onset or progression of clinical symptomatology.

In addition, treatment may be instituted in an attempt to prevent or restrict the long-term complications of PCOS, namely diabetes and its related complications, including cardiovascular disease. Currently the most effective modalities appear to be life-style modification and ovarian suppression by oral contraceptives

## Discussion

Our data showed that serum homocysteine levels were significantly higher in PCOS women than controls. Our findings are consistent with a previous smaller study by Battaqlia et al. (30). Morever in our review on 13 studies, with the exception of Mancini and Yilmaz, the same results were noted (20). Homocysteine has a well-known role in cardiovascular morbidity and mortality. It has primary atherogenic and prothrombotic properties. Homocysteine promotes leukocyte recruitment by upregulating monocyte chemoattractant protein-1 and interleukin-8 expression and secretion. The metabolite of homocysteine can combine with LDL-cholesterol to produce foam cells and atherosclerotic plaques. Homocysteine increases smooth muscle cell proliferation and enhances collagen production. Prothrombotic effects of homocysteine include attenuation of endothelial cell tissue plasminogen activator binding sites, activation of factor VIIa and V, inhibition of protein C and heparin sulfate, increased fibrinopeptide A and prothrombin fragments 1 and 2, increased blood viscosity, and decreased endothelial antithrombotic activity due to changes in thrombomodulin function.

Free radicals formed during the oxidation of reduced homocysteine may directly injured endothelial cells. Marked platelet aggregation may be secondary to the pro-aggregatory effects of homocysteine. Prolonged exposure of endothelial cells to homocysteine impairs the production of nitric oxide. Hyperhomocysteinemia has been linked to myocardial infarction and recurrent coronary events, adverse outcomes after angioplasty, carotid artery stenosis, recurrent venous thrombosis, osteoporosis, dementia and silent brain infarct (31, 32). The levels of homocysteine in the PCOS population compared with controls have been studied with conflicting results.

In multiple logistic regression analysis, age and BMI were not predictors of hyperhomocysteinmia. Because of the higher rate of hyperhomocysteinemia in PCOS subjects with significantly elevated fasting insulin, we suggest that it may be secondary to the higher prevalence of insulin resistance in PCOS patients (33).

Badawy et al. in their prospective case-control study on ninety PCOS women which used a cut off level of 11 µmol/L for a normal homocysteine level, found that 41.1% of PCOS patients and 2.9% of the control group had high homocysteine levels, which demonstrated the effect of insulin resistance on homocysteine levels (23). Kilic-Okman in a study of 29 patients with PCOS showed significant measures between the groups (33). In another similar study, the authors found that mean plasma homocysteine levels were significantly higher in the insulin-resistant PCOS patients as compared with non-insulin-resistant PCOS patients (34-37). Mancini et al. in their prospective case-control study on 44 patients showed that homocysteine levels did not differ among PCOS women and controls. In this study, they also assessed androgens, fasting glucose, insulin, leptin, fibrinogen, homocysteine, endothelin-1 and flow-mediated dilatation of the brachial artery to investigate their relationships to weight and PCOS. These researchers have suggested that weight and PCOS were two independent variables which have an effect on endothelial function (18). The lack of uniformity in the definition of PCOS and hyperhomocysteinemia as well as information on other cofactors such as BMI, fat distribution, and insulin sensitivity can explain the differences between our findings and other studies. To limit the potential confounding effect of disparate BMI between PCOS cases and controls, we adjusted for these differences in whole-group comparisons.

## Conclusion

In conclusion the findings of the study show significantly elevated levels of both lipid profile and homocysteine as cardiovascular risk markers in PCOS women. The various parameters like Serum TC, TG, LDL, VLDL, TC/HDL ratio, LDL/HDL ratio and TG/HDL ratio, homocysteine and CRP levels were elevated in the study group and also there was a reduction in the HDL levels in the study group. At the same time elevated inflammatory risk markers CRP and homocysteine can cause endothelial dysfunction and thus increase the risk of early onset cardiovascular risk in these young women. Hence correction of these CV risk factors in PCOS women can play an important role in decreasing the cardiovascular mortality in these patients. Hence, routine screening for these parameters helps in early identification of these cardiovascular risk factors can prevent the development of endothelial dysfunction, which is a reversible

early event in atherosclerosis and appropriate treatment should be aimed to control these parameter.

Based on early recognition of PCOS, efforts may be done to limit or forestall the onset or progression of clinical symptomatology. In addition, treatment may be instituted in an attempt to prevent or restrict the long-term complications of PCOS namely diabetes and its related complications, including cardiovascular disease. Currently the most effective modalities appear to be life-style modification and ovarian suppression by oral contraceptives..

## REFERENCES


1. Haugaard LK, Vestergaard H, Skouby SO. Polycystic ovary syndrome and comorbidity. Ugeskr Laeger. 2010;172(3):199–202. [PubMed]
2. Mak W, Dokras A. Polycystic ovarian syndrome and the risk of cardiovascular disease and thrombosis. Semin Thromb Hemost. 2009;35(7):613–620. [PubMed]
3. Salehpour S, Aghaee A. The effect of ovarian drilling on the fertility of patients with PCOS. Pajohand. 1999;4(20):423–428.
4. Salehpour S, Saadt N, Ashrafi M, Emami H. The effect of metformin therapy on menstural function in PCOS. Med J Iran Hosp. 2002;15(1):20–22.
5. Wild S, Pierpoint T, McKeigue P, Jacobs H. Cardiovascular disease in women with polycystic ovary syndrome at long-term follow-up: a retrospective cohort study. Clin Endocrinol (Oxf) 2000;52(5):595–600.[PubMed]
6. Uland PM, Refsom H. Plasma homocysteine, a risk factor for vascular diseases. J Lab Clin Med. 1989;114(5):473–501. [PubMed]
7. Dahlgren E, Janson PO, Johasson S, Lapidus L, Oden A. Polycystic ovary sandrome and risk for myocardial infarction.Evaluated from a risk factor model based on a prospective population study of women. Acta Obstet Gynecol Scand. 1992;71(8):599–604. [PubMed]
8. Talbott E, Guzick D, Clerici A, Berga S, Detre K, Weimer K, et al. Coronary heart disease risk factors in women with polycystic ovary syndrome. Arterioscler Thromb Vasc Biol. 1995;15(7):821–826. [PubMed]
9. Birdsall MA, Farquhar CM, White HD. Association between polycystic ovaries and extent of coronary artery disease in women having cardiac catheterization. Ann Intern Med. 1997;126(1):32–35. [PubMed]
10. Kelly CC, Lyall H, Petrie JR, Gould GW, Connell JM, Sattar N. Low grade chronic inflammation in women with polycystic ovarian syndrome. J Clin Endocrinol Metab. 2001;86(6):2453–2455. [PubMed]
11. Baccarelli A, Zanobetti A, Martinelli I, Grillo P, Hou L, Lanzani Get al. Air pollution, smoking and plasma homocysteine. Environ Health Prespect. 2007;115(2):176–181. [PMC free article] [PubMed]
12. Cabarkapa V, Stosić Z, Zeravica R, Ilincić B. The importance of homocysteine measurement in chronic renal failure. Med Preg. 2007;60(suppl 2):81–83. [PubMed]
13. Dierkes J, Westphal S, Luley C. The effects of fibrates and other lipid lowering drugs on plasma homocysteine levels. Expert Opin Drug Saf. 2004;3(2):101–111. [PubMed]
14. Rosenson RS. Antiatherothrombotic effects of nicotinic acid. Atherosclerosis. 2003;171(1):87–96.[PubMed]
15. Orio F Jr, Palomba S, Di Biase S, Colao A, Tauchmanova L, Savastano S, et al. Homocysteine levels and C677T polymorphism of methylenetetrahydrofolate reductase in women with PCOS. J Clin Endocrinol Metab. 2003;88(2):673–679. [PubMed]
16. D'Angelo A, Coppola A, Madonna P, Fermo I, Pagano A, Mazzola G, et al. The role of vitamin B12 in fasting hyperhomocysteinemia and its interaction with the homozygous C677T mutation of the methylenetetrahydrofolate reductase (MTHFR) gene.A case-control study of patients with early-onset thrombotic events. Thromb Heamost. 2000;83(4):563–570. [PubMed]
17. Vermeulen EG, Stehouwer CD, Twisk JW, van den Berg M, de Jong SC, Mackaay AJ, et al. Effect of homocysteinelowering treatment with folic acid plus vitamin B6 on progression of subclinical atherosclerosis: a randomised, placebo- controlled trial. Lancet. 2000;355(9203):517–522. [PubMed]
18. Mancini F, Cianciosi A, Reggiani GM, Facchinetti F, Battaglia C, de Aloysio D. Endothelial function and its relationship to leptin, homocysteine, and insulin resistance in lean and overweight eumenorrheic women and PCOS patients: a pilot study. Fertil Steril. 2009;91(6):2537–2544. [PubMed]
19. Oktem M, Ozcimen EE, Uckuyu A, Esinler I, Pamuk B, Bayraktar N, et al. Polycystic ovary syndrome is associated with elevated plasma soluble CD40 ligand, a marker of coronary artery disease. Fertil Steril. 2009;91(6):2545–2550. [PubMed]
20. Salehpour S, Taherzade P, Neisani Samani E. Leptin, Gherlin, Adiponectin, Homocysteine and Insulin resistance related to PCOS. International Journal of Fertility and Sterility (IJFS) 2008;3(3):101–104.
21. Yilmaz N, Pektas M, Tonguc E, Kilic S, Gulerman C, Gungor T, et al. The correlation of plasma homocysteine with insulin resistance in polycystic ovary syndrome. J Obstet Gynaecol Res. 2008;34(3):384–391. [PubMed]
22. Atamer A, Demir B, Bayhan G, Atamer Y, Ilhan N, Akkuş Z. Serum levels of leptin and homocysteine in women with polycystic ovary syndrome and its relationship to endocrine, clinical and metabolic parameters. J Int Med Res. 2008;36(1):96–105. [PubMed]
23. Badawy A, State O, El Gawad SSh, El Aziz OA. Plasma homocysteine and PCOS: the missed link. Eur Obstet Gynecol Reprod Biol. 2007;131(1):68–72. [PubMed]
24. de la Calle M, Gallardo T, Diestro MD, Hernanz A, Pérez E, Fernández-Miranda C. Increased homocysteine levels in polycystic ovary syndrome. Med Clin. 2007;129(8):292–294. [PubMed]
25. Yilmaz M, Biri A, Bukan N, Karakoç A, Sancak B, Törüner F, et al. Levels of lipoprotein and homocysteine in nonobese and obese patients with polycystic ovary syndrome. Gynecol Endocrinol. 2005;20(5):258–263. [PubMed]
26. Guzelmeric K, Alkan N, Pirimoglu M, Unal O, Turan C. Chronic inflammation and elevated homocysteine levels are associated with increased body mass index in women with polycystic ovary syndrome. Gynecol Endocrinol. 2007;23(9):505–510. [PubMed]
27. Loverro G, Lorusso F, Mei L, Depalo R, Cormio G, Selvaggi L. The plasma homocysteine levels are increased in polycystic ovary syndrome. Gynecol Obstet Invest. 2002;53(3):157–162. [PubMed]
28. Yarali H, Yildirir A, Aybar F, Kabakçi G, Bükülmez O, Akgül E, et al. Diastolic dysfunction and increased serum homocysteine concentrations may contribute to increased cardiovascular risk in patients with polycystic ovary syndrome. Fertil Steril. 2001;76(3):511–516. [PubMed]
29. The Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS) Hum Reprod. 2004;19(1):41–47. [PubMed]
30. Battaglia C, Mancini F, Cianciosi A, Busacchi P, Facchinetti F, Marchesini GR, et al. Vascular risk in young women with polycystic ovary and polycystic ovary syndrome. Obstet Gynecol. 2008;111(2 Pt 1):385–95. [PubMed]
31. Welch GN, Loscalzo J. Homocysteine and atherothrombosis. N Engl J Med. 1998;338(15):1042–1050.[PubMed]
32. Arnesen E, Refsum H, Bønaa KH, Ueland PM, Førde OH, Nordrehaug JE. Serum total homocysteine and coronary heart disease. Int J Epidemiol. 1995;24(4):704–709. [PubMed]
33. Kilic-Okman T, Guldiken S, Kucuk M. Relationship between homocysteine and insulin resistance in women with polycystic ovary syndrome. Endocr J. 2004;51(5):505–508. [PubMed]
34. Wijeyaratne CN, Nirantharakumar K, Balen AH Barth JH, Sheriff R, Belchetz PE. Plasma homocysteine in PCOS: dose it correlate with insulin resistance and ethnicit? Clin Endocrinoln. 2004;60(5):560–567. [PubMed]
35. Schachter M, Raziel A, Friedler S, Strassburger D, Bern O, Ron-El R. Insulin resistance in patients with polycystic ovary syndrome is associated with elevated plasma homocysteine. Hum Reprod. 2003;18(4):721–727. [PubMed]
36. McCarty MF. Insulin secretion as a potential determinant of homocysteine levels. Med Hypotheses. 2000;55(5):454–455. [PubMed]
37. Lo JC, Feigenbaum SL, Yang J, Pressman AR, Selby JV, Go AS. Epidemiology and adverse cardiovascular risk profile of diagnosed polycystic ovary syndrome. J Clin Endocrinol Metab. 2006;91(4):1357–1363. [PubMed]
38. Bickerton AS, Clark N, Meeking D, Shaw KM, Crook M. Lumb PEt alCardiovascular risk in women with polycystic ovarian syndrome (PCOS) J Clin Pathol. 2005;58(2):151–154. [PMC free article][PubMed]
39. Shroff R, Kerchner A, Maifeld M, Van Beek EJ, Jagasia D, Dokras A. Young obese women with polycystic ovary syndrome have evidence of early coronary atherosclerosis. J Clin Endocrinol Metab. 2007;92(12):4609–4614. [PubMed]
40. Alexander CJ, Tangchitnob EP, Lepor NE. Polycystic ovary syndrome: a major unrecognized cardiovascular risk factor in women. Rev Obstet Gynecol. 2009;2(4):232–239. [PMC free article][PubMed]