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Original Article

Biochemistry Section

Status of Homocysteine in Polycystic Ovary Syndrome (PCOS)

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ABSTRACT

Background: Polycystic ovary syndrome (PCOS) is the most common endocrine disease in women of reproductive age and is estimated to affect 5-10 % of the population. Women with PCOS have a clustering of cardiovascular risk factors, such as obesity, dyslipidemia, impaired glucose tolerance and hypertension. Homocysteine has been recognized recently as a risk factor for cardiovascular diseases. Preliminary investigations suggest that high sensitivity C-reactive protein, homocysteine and adiponectin are abnormal in women with PCOS. The possible determinants of elevated homocysteine concentration are still debated among authors who found significant correlations between homocysteine and insulin resistance or hyperandrogenism.

Aim: The purpose of this study is to evaluate homocysteine levels in the PCOS population compared with controls.

Materials and Methods: Study group comprised of 142 women with PCOS and 65 healthy non-PCOS controls. Body mass index (BMI), Waist circumference and serum homocysteine were measured in PCOS subjects and age matched controls.

Statastical Analysis: All values are expressed as mean ± SD. The results obtained are analysed statistically using the unpaired student t-test to evaluate the significance of differences between the mean values.

Results: The mean BMI, Waist circumference and serum homocysteine values are significantly increased in PCOS subjects when compared with non PCOS controls.

Conclusion: The present study has demonstrated increase in mean serum homocysteine concentrations in women with PCOS.

Keywords: Polycystic ovary syndrome, Homocysteine, Cardiovascular disease

INTRODUCTION

PCOS is the most common endocrine disease in women of reproductive age and is estimated to affect 5-10 % of the population [1]. Prominent features of the syndrome include menstrual dysfunction, infertility, elevated androgen levels and insulin resistance [1, 2]. Women with PCOS have a clustering of cardiovascular risk factors, such as obesity, dyslipidemia, impaired glucose tolerance and hypertension. Preliminary investigations suggest that serum biomarkers of cardiovascular disease such as high sensitivity C-reactive protein, homocysteine and adiponectin are abnormal in women with PCOS [3,4]. Homocysteine is an amino acid formed by the conversion of methionine to cysteine. It is metabolized by transsulfuration and remethylation.

Recent data have shown an increased prevalence of cardiovascular disease [5] and higher cardiovascular morbidity in women with PCOS [6,7]. Framingham Offspring Study has demonstrated, that hyperhomocysteinemia is associated with hyperinsulinemia and may partially account for increased risk of CVD associated with insulin resistance [8]. Insulin inhibits the hepatic cystathionine β synthetase activity, which increases serum homocysteine. Plasma homocysteine levels have been shown to correlate with blood pressure [9] and BMI [10]. Homocysteine is thought to impair implantation by interfering with the endometrial blood flow and its vascular integrity, which may contribute to early miscarriage [11,12].

The possible determinants of elevated Homocysteine concentration are still debated among authors who found significant correlations between Homocysteine and insulin resistance or hyperandrogenism [13-15]. The purpose of this study is to evaluate homocysteine levels in the PCOS population compared with controls.

MATERIALS AND METHODS

The present study was carried out in department of biochemistry, Mamata Medical College and General Hospital, Khammam,India. The study was approved by Institutional human ethical committee. Informed consent was obtained from the patients selected. One hundred forty two subjects with PCOS were taken as the cases from the department of obstrectics and gynaecology unit of Mamata General Hospital, Khammam and Syamala hospitals, Khammam, Andhra Pradesh, India. Sixty five age and sex matched healthy individuals are taken as the controls [Table/Fig-1]. Controls included were volunteers from relatives of the patients, staff and students. The diagnosis was based on Rotterdam criteria [16]. Body weight, height and waist circumference were measured and BMI was calculated as weight (kg) divided by height in square meter (m^2). Waist circumference is a measure of abdominal or centralized obesity, and is taken as midpoint between the lower margin of last palpable rib and top of iliac crest. Homocysteine was measured using Axix Homocysteine Enzyme Immunoassay (EIA).

Five ml of blood was collected from the subjects as well as controls after overnight fasting (12 hours) by venipuncture.

PCOS cases were categeorised into normal (BMI<23), overweight (BMI 23-25) and obese (BMI≥25). Patients were divided into two groups based on the BMI, subgroup 1 consisting of patients with normal BMI (<23) and subgroup 2 consisting of patients with increased BMI (≥23)(overweight+obese). Based on waist circumference, PCOS were categorized into two groups, normal cases (waist<80) and obese cases (waist≥80). Controls were also sub categorized based on BMI and waist.

Study design: Cross sectional comparative study

Inclusion Criteria: All the patients in the age group of 20-35 were

diagnosed for PCOS using Rotterdam criteria. Subjects with normal kidney function were included.

Exclusion Criteria: The subjects having Diabetes mellitus, Hypertension, Coronary heart disease and endocrine disorders were excluded. Alcoholics, smokers, pregnant women, subjects on vitamin supplementation and subjects with altered kidney function (random urinary protein >16mg/dl, serum creatinine >1.1mg/dl) are also excluded from the study.

STATISTICAL ANALYSIS

All values were expressed as mean ± SD. The results obtained were analysed statistically using the unpaired student 't' test to evaluate the significance of differences between the mean values.

RESULTS

Prevalence of obesity is more in PCOS cases compared to controls. When compared with BMI central obesity is more pronounced in PCOS cases [Table/Fig-1]. The mean BMI and waist circumference were increased in PCOS cases when compared to controls. Serum homocysteine is increased in PCOS cases when compared with controls [Table/Fig-2].

Cases (n=142)	Controls (n=65)
Based on BMI	
Normal cases (n=61): 43% (<23) Overweight cases (n=26): 18% (23-25) Obese cases (n=55): 39% (≥ 25)	Normal controls(n=32): 49% (<23) Overweight controls(n=20): 31% (23-25) Obese controls(n=13): 20% (≥ 25)
Based on waist	
Normal cases (n=58): 41% (<80) Obese cases (n=84): 59% (≥ 80)	Normal cases (n=34): 52% (<80) Obese cases (n=31): 48% (≥ 80)

[Table/Fig-1]: Prevalence of obesity in cases and controls

Parameter	Mean ±SD (Total Controls)	Mean ±SD (Total Cases)	P-value
BMI (kg/m2)	24.14 ±5.20	24.31 ± 4.42	0.8153
Waist (cm)	79.95 ±9.90	82.07±9.47	0.1430
Homocysteine (µmol/L)	7.13 ±2.32	10.13 ±2.80	<0.0001*

[Table/Fig-2]: Mean ±SD and p values of various biochemical parameters in PCOS cases (n=142) and non PCOS controls (n=65)
*Statastically significant.

Based on BMI

There was an incremental increase of serum homocysteine from normal, overweight and obese cases when compared with their respective controls. The increase was not significant in the overweight categeory [Table/Fig-3]. PCOS cases were divided into two subgroups based on BMI: Subgroup 1 with normal BMI(<23) and subgroup 2 with increased BMI(≥23), homocysteine is increased with increase in BMI [Table/Fig-4].

Parameter	Normal controls vs normal cases	Overweight controls vs overweight cases	Obese controls vs obese cases
Homocysteine	6.78±1.81 vs 8.95 ± 2.21 *	8.55±3.17 vs 9.31±1.61NS	6.78±2.18 vs 11.89±3.01*

[Table/Fig-3]: (Mean ±SD) of normal controls (n=32) and normal cases (BMI<23) (n=61), overweight controls (n=13) and overweight cases (n=26)(BMI 23-25) obese controls (n=20) and obese cases (n=55) (BMI ≥ 25)
*Statastically significant, NS, not significant.

Parameter	(Subgroup 1 BMI<23)	(Subgroup 2 BMI≥ 23)	p-value
BMI	20.4452 ±1.8778	27.2299 ±3.4383	<0.0001*
Homocysteine	8.9579 ±2.2109	11.0190 ±2.8894	<0.0001*

[Table/Fig-4]: Sub group 1: Normal BMI (BMI<23) (n=61): vs Sub group 2: increased BMI (overweight + obese) (BMI ≥ 23) (n= 81) (Mean ±SD)
*Statastically significant.

Based on Waist

Based on waist cases were divided into two subgroups: Normal and obese; there was significant increase in the mean values [Table/Fig-5]. When comparision was made between subgroups based on waist: normal controls, normal cases, obese controls, and obese cases, significant increase of mean serum homocysteine level was observed in normal and obese cases compared with respective controls. Significant incremental increase of serum homocysteine was observed from normal controls, obese controls, normal cases and obese cases [Table/Fig-6].

Parameter	Normal cases Waist < 80)	(Obese cases Waist ≥ 80)	P-value
Waist	73.2414 ±6.0533	88.1667 ±5.9492	<0.0001*
Homocysteine	8.9195 ±2.1000	10.9719 ±2.9311	<0.0001*

[Table/Fig-5]: Normal cases(<80) (N=58) and obese cases (≥ 80) (N=84): (Mean ±SD)
*Statastically significant.

Parameter	Normal Controls	Normal cases	Obese Controls	Obese cases
Waist	72.85±4.678	73.24±6.05	87.74±8.075	88.16±5.94
Homocysteine	6.49±1.8844	8.919±2.10*	7.84±2.5792	10.97±2.93*

[Table/Fig-6]: Comparision based on waist: normal controls, normal cases (< 80), obese controls and obese cases (≥ 80) (Mean ±SD)
*Statastically significant.

DISCUSSION

Homocysteine has been recognized recently as a risk factor for cardiovascular diseases. Our data showed that serum homocysteine levels were significantly higher in PCOS women than controls. Our findings are consistent with a previous study by Loverro et al., [17] and Badawy et al., [18]. Mancini et al., [19] in their study found no significant difference in homocysteine levels among PCOS women and controls.

Studies by Ilhan Tarkun et al., have shown correlation between homocysteine and BMI [20]. Schachter et al., [13], has shown correlation between homocysteine and insulin resistance. Mohan SK. and Priya V V have found that increased homocysteine levels and decreased antioxidant capacity may contribute to the increased risk of cardiovascular disease in women with PCOS [21]. Sagar Salehpour et al., have evaluated homocysteine levels in PCOS subjects based on BMI matched subjects. In our study homocystiene levels were compared among PCOS cases and controls as well as in subgroups based on BMI and waist circumference [22].

In our study mean serum homocysteine levels showed significant increase in PCOS cases. The increase was more pronounced with increase in BMI and waist. Higher levels were observed in obese cases when compared with normal cases and controls. The increasing global prevalence of obesity may play a key role in promoting the development of PCOS in susceptible individuals. In addition, there is no doubt that obesity aggravates preexisting clinical, hormonal and metabolic features in most women with PCOS [23].

Homocysteine has a well-known role in cardiovascular morbidity and mortality with its atherogenic and prothrombotic properties. Molecular mechanisms of homocysteine-induced cellular dysfunction include increased inflammatory cytokine expression, induction of oxidative stress, activation of apoptosis, defective methylation [24].

The metabolite of homocysteine can combine with LDL-cholesterol to produce foam cells and atherosclerotic plaques. Free radicals formed during the oxidation of reduced homocysteine may directly injure endothelial cells. Marked platelet aggregation may be secondary to the pro-aggregatory effects of homocysteine. Prolonged exposure of endothelial cells to homocysteine impairs production of nitric oxide. Hyperhomocysteinemia has been linked to myocardial infarction and recurrent coronary events.

Homocysteine promotes leukocyte recruitment by upregulating monocyte chemoattractant protein-1 and interleukin-8 expression and secretion. 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 [22].

CONCLUSION

Our study has demonstrated that mean serum homocysteine concentrations are increased in women with PCOS. The increase was more pronounced with increase in BMI and waist. Higher levels were observed in obese cases when compared with normal cases and controls. There is a growing body of evidence demonstrating disturbed Homocysteine metabolism in PCOS women. As PCOS is associated with various factors like insulin resistance, obesity, oxidative stress, dyslipidemia which are inturn influenced and agrravated by hyperhomocysteinemia may have more pronounced risk. Further studies are required to clarify the role of homocysteine in human reproductive physiology and to elucidate the mechanism to link these factors and aim at better treatment for PCOS to prevent short term as well as long term complications.

Screening for homocysteine status may be beneficial. Vitamin supplementation (folic acid, B6, B12) can improve pregnancy outcome and can reduce cardiovascular risk. The results of our study emphasize the need for initiating life style measures early and in the overweight category itself. This will supplement PCOS treatment and can help in management of PCOS.

REFERENCES

[1] Franks S. Polycystic ovary syndrome. *N Engl J Med.* 1995;333(13):853-61.
[2] Dunaif A. Insulin resistance in polycystic ovarian syndrome. *Ann N Y Acad Sci.* 1993;687:60-4.
[3] 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.
[4] 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):821-26.
[5] Wild RA. Obesity, lipids, cardiovascular risk and androgen excess. *Am J Med*. 1995;98(1A):27S-32S.
[6] Homocysteine Studies Collaboration. Homocysteine and risk of ischemic heart disease and stroke: a meta-analysis. *JAMA.* 2002;288(16):2015–22.
[7] Pierpoint T, McKeigue PM, Isaacs AJ, Wild SH, Jacobs HS. Mortality of women with polycystic ovary syndrome at long-term follow-up. *J Clin Epidemiol* 1998;51(7):581-86.
[8] Framingham Offspring Study. Fasting plasma homocysteine levels in the insulin resistance syndrome: the Framingham offspring study. *Diabetes Care* 2001;24(8):1403–10.
[9] Malinow MR, Levenson J, Giral P, Nieto FJ, Razavian M, Segond P, et al. Role of blood pressure, uric acid, and hemorheological parameters on plasma homocyst(e)ine concentration. *Atherosclerosis.* 1995;114(2):175-83.
[10] Giltay EJ, Hoogeveen EK, Elbers JM, Gooren LJ, Asscheman H, Stehouwer CD. Insulin resistance is associated with elevated plasma total homocysteine levels in healthy, non-obese subjects. *Atherosclerosis.* 1998;139(1):197-8.
[11] Nelen WLDM, Blom HJ, Steegers EAP, Den Heijer M, Eskes TAKB. Hyperhomocysteinemia and recurrent early pregnancy loss: a metaanalysis. *Fertil Steril.* 2000;74:1196–9.
[12] Quere I, Mercier E, Bellet H, Janbon C, Mares P, Gris JC. Vitamin supplementation and pregnancy outcome in women with recurrent pregnancy loss and homocysteinemia. *Fertil Steril.* 2001;75:823–5.
[13] 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;18:721–27.
[14] Wijeyaratne CN, Nirantharakumar K, Balen AH, Barth JH, Sheriff R, Belchetz PE. Plasma homocysteine in polycystic ovary syndrome: does it correlate with insulin resistance and ethnicity? *Clin Endocrinol (Oxf).* 2004;60:560–67.
[15] Yilmaz N, Pektas M, Tonguc E, Kilic S, Gulerman C, Gungor T, Mollamahmutoglu L. The correlation of plasma homocysteine with insulin resistance in polycystic ovary syndrome. *J Obstet Gynaecol Res.* 2008;34:384–91.
[16] 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;19:41–47.
[17] Loverro G, I orusso F,Mei L, Depalo R, Cormio G, Selvaggi L. The plasma homocysteine levels are increased in polycystic ovary syndrome. *Gynecol obstet Invest*. 2002;53:157-62.
[18] Ahmed Badawy, Omnia State, Soma ShAbd ElGawad and Omar Abd El Aziz. "Plasmahomocysteine and polycystic ovary syndrome: themissed link". *European Journal of Obstetrics and Gynaecology and Reproductive Biology*. 2007;131(1):68– 72.
[19] 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):2537-44.
[20] Ihan Tarkun, Berrin Çetinarslan, Zeynep Cantürk, Erdem Türemen. The Plasma Homocysteine Concentrations and Relationship with Insulin Resistance in Young Women with Polycystic Ovary Syndrome. *Turkish Journal of Endocrinology and Metabolism*. 2005; 1 : 23-28.
[21] Mohan SK and Priya VV. Lipid peroxidation, glutathione, ascorbic acid, vitamin E, antioxidant enzyme and serum homocysteine status in patients with polycystic *ovary syndrome Biology and Medicine*. 2009; 1(3):44-49.
[22] Saghar Salehpour, Ozra Manzor-al-ajdad, Elham Neisani Samani, Alireza Abadi. Evaluation of Homocysteine Levels in Patients with Polycystic Ovarian Syndrome. Roya Institute. *International Journal of Fertility and Sterility*. 2011; 4(4): 168-71
[23] Pasquali R, Gambineri A, Pagotto U. The impact of obesity on reproduction in women with polycystic ovary syndrome. *BJOG*. 2006;113:1148-59
[24] Forges T, Monnier-Barbarino P, Alberto JM, Gueant-Rodriguez RM, Daval JL, Gueant JL. Impact of folate and homocysteine metabolism on human reproductive health. *Hum Reprod Update.* 2007;13:225–38.

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