

ORIGINAL ARTICLE

Evaluation of Plasma Homocysteine Levels in Patients of PCOS

Amita Diwaker¹, Dhiraj Kishore^{2*}**Abstract**

Background: Homocysteinemia in PCOS may impair implantation by interfering with endometrial blood flow and has been documented to increase the adverse pregnancy outcome.

Aims: The objective was to evaluate the relationship between insulin resistance and serum homocysteine in subjects with polycystic ovarian syndrome (PCOS).

Material and Methods: Cross sectional Case Control observational study done in Department of Obstetrics and Gynecology, KGMU Lucknow. Cases were 50 PCOS women as a study group and 40 women with infertility due to isolated male cause as a control group. Serum homocysteine levels compared in PCOS patients.

Results: Mean homocysteine raised in cases ($11.8 \pm 5.5 \mu\text{mol/L}$) than control ($7.8 \pm 2.2 \mu\text{mol/L}$), $p < 0.001$ Considering $11 \mu\text{mol/l}$ cut off level for normal homocysteine, 36% of PCOS patients (18 of 50) and 10% of control (4 out of 40) had high homocysteine levels, $p < 0.001$. 8% of PCOS patients without insulin resistance (4 out of 50) had a high homocysteine level, while 28% of PCOS patients with insulin resistance (14 out of 50) had homocysteinemia. Mean plasma homocysteine level was very high in insulin resistant case group subjects ($13.9 \pm 5.6 \mu\text{mol/L}$) than non insulin resistant subjects in case group (8.2 ± 2.7), $p < 0.001$

Conclusion: Insulin resistance and hyperinsulinaemia in patients with PCOS is associated with elevated plasma homocysteine This finding may have important implications in the short-term reproductive outcome, and the long-term cardiovascular complications associated with insulin-resistant PCOS.

Introduction

Polycystic ovary syndrome is a common endogynecological disorder of reproductive age group with prevalence of 5-10%, having characteristics of menstrual irregularities, biochemical and clinical hyperandrogenism as hirsutism, seborrhea, acne and hyperinsulinemia. Recently homocysteinemia is been in debate as a independent risk factor for disease related early and late complication in patients of PCOS along with other pro inflammatory marker due to insulin resistance state and hyperandrogenism.¹ Homocysteinemia is classified as moderate $11-30 \mu\text{mol/L}$, intermediate $31-100 \mu\text{mol/L}$ and a value above $100 \mu\text{mol/L}$ is severe homocysteinemia.² Homocysteinemia has been documented to increase the probability of early pregnancy loss due to impaired implantation by interfering with endometrial

blood flow and vascular integrity. Insulin resistance or insulin resistance syndrome (metabolic syndrome) itself is risk factor for cardiovascular disease, hypertension, diabetes, nephropathy and dyslipidemia. All of these long term implication of this metabolic syndrome can be aggravated by elevated plasma homocysteine levels in patients of PCOS.³ So this probable relation between increased serum homocysteine and insulin resistance in women with PCOS might provide a clue regarding vascular complications in women with PCOS. In accordance with these facts, the present study has been designed to assess the correlation of plasma homocysteine levels in Polycystic ovarian syndrome women and also aims to assess the relationship

between homocysteine levels and insulin resistance in women with or without Polycystic ovary syndrome.

Material and Methods

This Cross section observational study was carried out in the Department of Obstetrics and Gynecology in collaboration with the Department of Pathology and Department of Medicine, KGMU and included a total of 90 women. There were 50 subjects in case group i.e. women having polycystic ovarian syndrome according to Rotterdam criteria⁴ and age matched 40 women included in control group who attended infertility clinic during the same period, due to isolated male factor. Women receiving metformin, folic acid, methylcobalamine and infertility treatment were excluded. Both the cases and control population were studied for variables like fasting blood glucose levels in mg/dl, fasting insulin levels in $\mu\text{IU/ml}$. Homeostasis model assessment of insulin resistance (HOMA-IR) ≥ 2.5 was considered as indicative of insulin resistance [Glucose (mg/dl) \times insulin ($\mu\text{IU/ml}$) /405].⁵⁻⁷ Serum homocysteine $\geq 11 \mu\text{mol/l}$ was considered as indicative of homocysteinemia.⁸⁻¹⁰ After selection of subjects 4ml of blood was collected slowly to avoid frothing and hemolysis after an overnight fast on the day two or three of menstruation. In our case we have done the test within 30 hours of collection of blood from the patient by using Fluorescence Polarisation Immunoassay (FPIA) method. In this study homocysteinemia was defined as $\geq 11 \mu\text{mol/L}$ in plasma. The data was analyzed to correlate the levels of plasma Homocysteine in women with or without polycystic ovarian syndrome. Also the relationship between homocysteine and insulin resistance in polycystic ovarian syndrome and the controls were looked for. Statistics were

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Table 1: Distribution of Subjects According to HOMA-IR Score and Homocysteinemia i.e. ($\geq 11 \mu\text{mol/L}$)

Plasma Homocysteine $\mu\text{mol/L}$	Case = 50 ($11.87 \pm 5.55 \mu\text{mol/L}$)			Control = 40 (7.80 ± 2.29)		
	IR (Homa IR >2.5)	NIR (Homa IR <2.5)	Total	IR (Homa IR >2.5)	NIR (Homa IR <2.5)	Total
Total	32	18	50	2	38	40
<11 (No)	18	14	32	0	36	36
≥ 11 (No)	14(77.7%)	4(22.3%)	18	2(50%)	2(50%)	4

HOMA IR $\chi^2=33.59$, $df=1$, $p<0.001$

Table 2a: Correlation between PCOS and Insulin resistance and homocysteinemia (*In control group only 2 subject had Insulin resistance)

	Case (PCOS)		Control (normal ovary)				Total	P value				
	.N.I.R	I.R	N.I.R		I.R							
Subjects	No	%	No	%	No	%	No	%	90			
	18	36	32	64	38	95	2*	5		56	62.2	34
Mean Plasma homocysteine \pm SD	8.24 ± 2.77		13.92 ± 5.69		7.80 ± 2.29		10.07 ± 4.83		F-23.99 Sig-0.000			
					Total mean control		Total mean case + control					

*could not be analyzed due to less number of subjects in this category and merged as a single group with N.I.R among control group

Table 2b: Multiple Comparisons for plasma homocysteine comparison among NIR (PCOS), IR (PCOS), and Control. **1 (NIR, PCOS), 2 (I.R, PCOS), 3 (control)

Dependent Variable	homagr	homagr	Mean difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
NIR vs. IR	1	2	-5.68601*	1.15737	.000	-7.9864	-3.3856
		3	.43106	1.11492	.700	-1.7850	2.6471
		2	5.68601*	1.15737	.000	3.3856	7.9864
IR vs. Control	3	1	6.11706*	.93166	.000	4.2653	7.9688
		2	-4.3106	1.11492	.700	-2.6471	1.7850
NIR vs Control	3	1	-4.3106	1.11492	.700	-2.6471	1.7850
		2	-6.11706*	.93166	.000	-7.9688	-4.2653

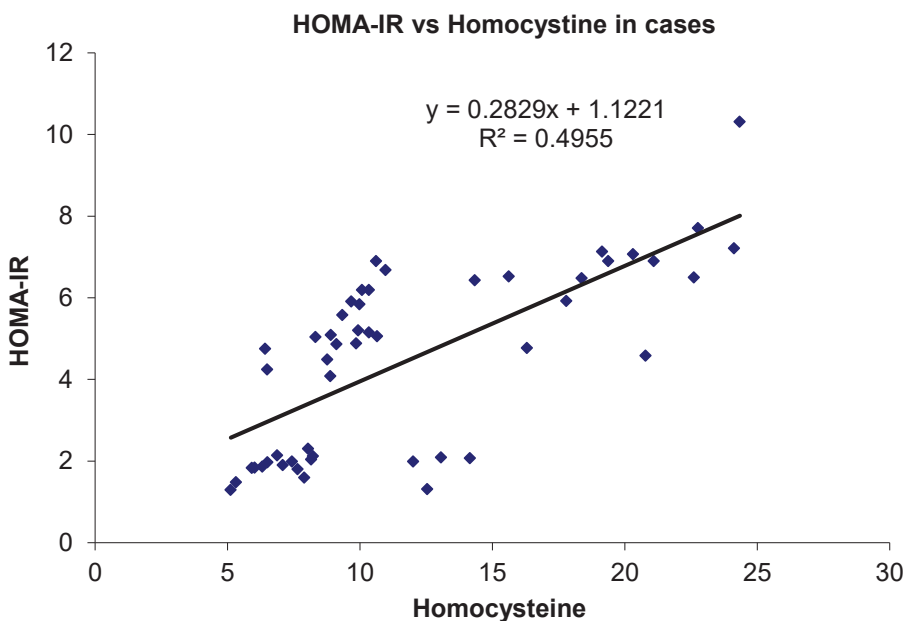


Fig. 1: Distribution of subjects according to HOMA IR values and plasma homocysteine level in cases

calculated with the aid of the SPSS 16. Chi-square test, independent Samples t-test, multiple comparison test were applied to look for significance of data: For the purpose of this study 95% confidence level has been chosen and corresponding 'p' value <0.05 has been

taken as significant.

Observation

Plasma homocysteine level was significantly raised in cases 18 (36%) with mean of ($11.87 \pm 5.55 \mu\text{mol/L}$) compared to control 4 (10%) with mean

of (7.80 ± 2.29), $p<0.001$. Prevalence of Insulin resistance (HOMA IR ≥ 2.5) was present in 64% of cases and considerably higher than control 5%, $p <0.001$ (Table 1).

The result strongly suggests insulin resistance have strong correlation for high plasma homocysteine in subjects of PCOS. Mean plasma homocysteine level was quite high in insulin resistant subject in case group (13.92 ± 5.69) than non insulin resistant subjects in case group (8.24 ± 2.77) and was statistically significant ($F=23.996$, $p <0.001$). Multiple comparison of mean plasma homocysteine level difference between insulin resistant and non insulin resistant subjects of case group was significant ($p <0.001$), between insulin resistant subjects of case group (13.92 ± 5.69) and control (7.80 ± 2.291) was also significant ($p <0.001$), but comparison between non insulin resistant subjects in case group and total (as 2 subjects were insulin resistant so all subjects were merged to form control group) control subjects was not significant ($p <0.7$). It simply suggests insulin resistance is a major risk factor for higher concentration of plasma homocysteine in patients of PCOS (Table 2a, 2b).

While analyzing the relationship between insulin resistance i.e. HOMA IR values and plasma homocysteine concentration in cases and control group, it was clear that there is a significant correlation between these two and the correlation coefficient was found to be quite significant ($R^2=0.495$, 0.7) in case group and insignificant in control group ($R^2=0.09$, 0.03). This suggests increase in the HOMA IR values increases plasma homocysteine level in cases and vice versa. The scattered points shows clustering of subjects on same value of HOMA IR in cases between 2 groups i.e. plasma homocysteine concentration <11 and >11 $\mu\text{mol/L}$ suggesting factors other than insulin resistance of this metabolic syndrome may be playing important role in development of homocysteinemia in patients of PCOS, that needed to be found out, however we have not investigated our subjects for MTHFR gene mutation (Figures 1 and 2).

Further we looked for separate relationship of value of HOMA IR and plasma homocysteine concentration in case group which showed significant

HOMA-IR vs Homocysteine in control

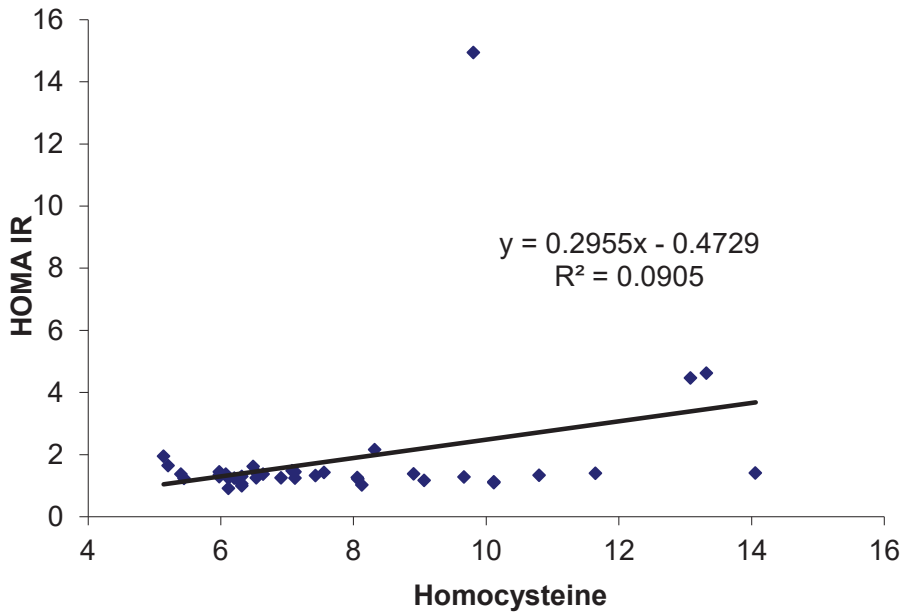


Fig. 2: Distribution of subjects according to HOMA IR values and plasma homocysteine level

Table 3a: Trend of HOMA IR score according to increasing level of homocysteine

Group of Homocysteine range	Plasma Homocysteine $\mu\text{mol/L}$	Case			Control		
		No	HOMA IR range	Mean HOMA IR	No	HOMA IR range	Mean HOMA IR
1	5-9.9	26 (52%)	1.29- 5.91	3.31 \pm 1.65	33 (82.5%)	0.91-4.04	2.38 \pm 0.67
2	10-14.9	11 (22%)	1.31- 6.90	4.55 \pm 2.21	7 (17.5%)	1.09- 4.62	2.20 \pm 1.60
3	15-19.9	6 (12%)	4.77- 7.13	6.29 \pm 0.85	0		0
4	\geq 20	7 (14%)	4.58- 10.31	7.18 \pm 1.70	0		0

difference among different categories of plasma homocysteine range in cases relating to HOMA IR values ($F=11.83$, $p < 0.001$). Further we attempted to see the group difference and found that plasma homocysteine above $15 \mu\text{mol/L}$ was present in 26% of case group compare to 0% in control group. Most of subject in control group had plasma homocysteine less than $10 \mu\text{mol/L}$ (82.5%). It was noted that with increasing level of plasma homocysteine concentration in case group, HOMA IR score is not increasing in similar ratio specially after Hcy level $\geq 20 \mu\text{mol/L}$. So we draw a inference that severity of insulin resistance is contributing to increased plasma homocysteine level only up to a extent after that there may be some other factor in PCOS responsible for homocysteinemia, however our sample size is very small and a bigger study is needed to draw a conclusive statement (Tables 3a, 3b).

Discussion

PCOS constitutes many adverse metabolic states in reproductive age

group leading to infertility and adverse pregnancy outcome, homocysteinemia is one of these adverse metabolic effect. Identification of homocysteinemia is quite important as it is a independent risk factor for obstetrical vascular syndrome like of spiral arteries abnormalities causing placental abruption, preeclampsia, maternal deep vein thrombosis.¹¹ Close association of insulin resistance and polycystic ovary syndrome, makes this endocrinopathy a complex adverse state for development of short term pregnancy related and long term systemic i.e. cardiovascular complication.^{12,13}

In this study, we looked for the association between insulin resistance and homocysteinemia in women with PCOS. Plasma homocysteine ranged from 5.12 to $24.34 \mu\text{mol/L}$ (11.87 ± 5.55) in cases and in control 5.05 to $14.06 \mu\text{mol/L}$ (7.80 ± 2.29). It was statistically significant ($t=4.3$, $\text{sig} < 0.001$) and similar findings were observed in other studies.¹ Many study have been done which suggested homocysteinemia a risk factor for many adverse clinical

Table 3b: p value of comparison of HOMA IR values among different group of plasma homocysteine range in cases

Comparison between group	p value
1 vs 2*	0.052 (just insignificant)
1 vs 3	0.000
1 vs 4	0.000
2 vs 3*	0.054 (just insignificant)
2 vs 4	0.003 (significant)
3 vs 4*	0.357 (insignificant)

situation is quite common in patients of PCOS.^{14,15}

The adverse vascular outcome in cases of PCOS is been correlated with plasma plasminogen activator inhibitor (PAI-1) concentration and this adverse obstetrical state due to elevated PAI-1 may be further aggravated by homocysteinemia.¹⁶ Endothelial dysfunction in PCOS was shown by decreased response to vasodilatation. As some studies concluded that homocysteinemia increases oxidative stress markers and increases concentration of endothelin-1 in insulin-resistant PCOS patients which correlates with development of late cardiovascular complication.¹⁷

In our study there was difference in plasma homocysteine level in case and control group so we tried to understand the pathophysiological changes in our case group and possible explanation of all observation. In the current study while looking for, the association of Insulin resistance in case group and homocysteinemia we found that insulin resistance is quite prevalent in case group compared to control group like previous studies. Hyperinsulinemia or any phenotypes of the IR syndromes in the general population may have various adverse metabolic effects, including increased level of plasma homocysteine.¹¹ This association was evaluated earlier in patients with PCOS and infertility, they found strong correlation between IR and elevated Hey.^{1,8}

Our study showed consistent results with previous one. The IR or the other associated syndrome itself is a risk factor for cardiovascular disease, the long-term implications of these metabolic syndromes may be aggravated by elevated homocysteine.^{18,19} So in metabolic terms, PCOS may be considered as a variant of the IRS or an early marker of the IRS. We are in view that management of infertility

must include investigations for these metabolic dysfunction, including raised plasma homocysteine level, and treatment should be aimed to correct these adverse metabolic factors in order to achieve the best results in the short term (reproductive function) and in the long term (cardiovascular and metabolic functions) complications. Methyltetrahydrofolate reductase (MTHFR) enzyme deficiencies or the vitamin B₁₂ levels and folic acid levels were examined in few study and no significant differences were found between PCOS and controls.²¹

In this study, strong association between insulin resistance in PCOS and increased serum homocysteine draws attention to PCOS as a marker for a possible insulin resistance syndrome and also it was noted (Table 3b) that with increasing level of homocysteine concentration in PCOS group HOMA IR score is not increasing in similar ratio specially after plasma homocysteine level $\geq 20 \mu\text{mol/L}$ i.e. moderate range of homocysteinemia. So we draw a inference that severity of insulin resistance is contributing to increased homocysteine level only up to a extent after that there may be some other factor in PCOS responsible for homocysteinemia which is needed to be investigated, however our sample size is quite small and a bigger study is needed to draw a conclusive statement. So in conclusion insulin resistance or insulin resistance syndrome prevalence is very high in patients of PCOS which have proven adversities on outcome of pregnancy and this is compounded by state of hyperhomocysteinemia, a possible component of these metabolic syndrome. It may be recommended that when PCOS is diagnosed, other

metabolic complications should be investigated. The aim should be targeted to solve short-term problems of PCOS, i.e. reproductive failure, without ignoring the serious, long term complications, i.e. cardiovascular and metabolic abnormalities. Further we recommend study with bigger sample size for the effect of insulin sensitizers on the level of homocysteine and their therapeutic implications are required.

Abbreviations

HOMA-IR- Homeostasis model assessment of insulin resistance; I.R- Insulin resistant; N.I.R- Non insulin resistant; PAI-1- Plasminogen activator inhibitor 1; Hcy- Homocysteine

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