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EVALUATION OF SERUM HOMOCYSTEINE LEVELS IN ASSOCIATION WITH LIPID PROFILE IN PATIENTS WITH POLYCYSTIC OVARY SYNDROME

KEY WORDS: polycystic ovary syndrome, homocysteine, lipid profile

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ABSTRACT

Aim: To evaluate serum Homocysteine level, lipid profile and an association between Homocysteine and component of lipid profile in patients with polycystic ovary syndrome.

Material & Methods: 50 female subjects diagnosed with PCOS were enrolled for the study based on inclusion and exclusion criteria visiting the outpatient department of Obstetrics and Gynaecology of Mahatma Gandhi Medical College and Hospital, Jaipur. 50 age matched females with regular menstruation cycle constituted the control group.

Observations: Levels of Homocysteine [P=0.000] were higher in the PCOS group. Levels of total cholesterol [P=0.000], triglycerides [P=0.000], LDL [P=0.000] and VLDL [P=0.000] were significantly higher in PCOS group whereas levels of HDL [P=0.000] were significantly lower. A significant correlation was also observed between homocysteine and total cholesterol [P=0.01] and homocysteine and LDL [P=0.04].

Conclusion: The present study has demonstrated that the levels of homocysteine, total cholesterol, LDL and VLDL are significantly higher and the level of HDL is lower in patients with polycystic ovary syndrome. This study also shows a positive correlation between homocysteine and total cholesterol and homocysteine and LDL-C. Both homocysteine and LDL-C are biomarkers of cardiovascular disease so; there correlation can be further studied with some more cardiac markers in PCOS patients.

The homocysteine levels are affected by several dietary factors such as vitamin B6, B12 and folic acid. The study therefore recommends further research on the association of homocysteine levels with concentration of vitamin B12 and folic acid in patients with polycystic ovary syndrome.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is an endocrine disorder which has affects on 10 to 15% of women of reproductive age group [1]. It's a heterogeneous disorder, with multiple generative, cosmetic and metabolic complexities that's characterized by dysfunction in ovulation and biochemical hyperandrogenism and also the presence of polycystic ovarian morphology [2].

Hirsutism, acne, and baldness are directly associated with elevated androgen levels, and also the prevalence of polycystic ovaries on pelvic ultrasound exceeds 70% in patients with PCOS [3].

In PCOS, there is elevation of luteinizing hormone (LH) and gonadotropin-releasing hormone (GnRH) levels, whereas follicular-stimulating hormone (FSH) levels are muted or unchanged. As a result of the increase in GnRH, stimulation of the ovarian thecal cells to produces more androgens [4]. Women with PCOS seem to have theca interna dysplasia, a thicker layer of the theca cells, which seem to be responsible for their increased androgen steroidogenesis [5].

Many studies show that more than 50% of patients with PCOS develop prediabetes or diabetes, and there is an increased risk of myocardial infarction (MI), dyslipidemia, hypertension, anxiety, depression, endometrial cancer, sleep apnea and cardiovascular disease [6]. PCOS will be represented as an oligogenic disorder during which the interaction of type of genetic and environmental factors confirm the heterogeneous, clinical, and biochemical phenotype [7]. Environmental factors shown in PCOS like obesity may be increased by poor dietary choices and physical inactivity [8]. The reproductive and metabolic features of PCOS are sometimes reversible with lifestyle modifications like weight loss and exercise [9].

The diagnosis of PCOS relies on the Rotterdam criteria for the presence of any two of the subsequent conditions:- (1) Chronic anovulation, (2) Clinical/biochemical parameters for hyperandrogenism, (3) Polycystic ovaries on ultrasonography[10].

PCOS has been linked to insulin resistance and obesity. Insulin helps to regulate ovarian perform, and also the ovaries reply to excess insulin by manufacturing androgens, which may cause anovulation [8]. Although insulin resistance is not a disease, its presence is related to increased risk of cardiovascular morbidity and mortality and type-2 diabetes [11]. Hyperinsulinemia additionally seems to be the most contributors to the increased cardiovascular risk of women with PCOS. It also decreases liver production of sex hormone binding globulin (SHBG) which increases the quantity of bioavailable free circulating sex steroids [12].

Homocysteine is an intermediate formed during the breakdown of the organic compound methionine, and will undergo remethylation to methionine, or transe-sulphuration to cystathione and cysteine [13]. Plasma homocysteine exist in varied fractions, total plasma homocysteine is that the total of protein bound, free oxidized and reduced species of homocysteine in plasma [14].

Insulin has inhibitory effect on enzyme hepatic cystathione beta synthase, which is involved in methionine metabolism. Insulin level is additionally introduced as a determining factor of Homocysteine levels. Insulin resistance seems to extend the Homocysteine levels [15].

In patients with PCOS cardiac biomarkers have not been yet studied much so, therefore evaluating homocysteine in these patients helpful in predicting cardiovascular risk. Homocysteine is a good biomarker of CVD as it damages the vascular endothelium directly and it is also related to an increased risk of ischemic heart disease and atherosclerosis [16]. Homocysteine is thought to impair implantation by interfering with the endometrial blood flow and its vascular integrity,

which may contribute to early miscarriage [17].

It was observed that females with hirsutism and oligomenorrhea had lower HDL-C and high triglycerides, suggesting an association between menstrual irregularities and Dyslipidemia [18].

Dyslipidemia may be a quite common metabolic abnormality in women with PCOS, with a prevalence of up to 70% [19]. Dyslipidemia is characterized by higher levels of total cholesterol, triglycerides, low density lipoprotein (LDL), very low density lipoprotein (VLDL) and lower level of high density lipoprotein (HDL). It is also a serious risk factor for cardiovascular disease in patients with PCOS [20].

Insulin resistance could be a key pathophysiology of PCOS, and its play a central role by stimulation of lipolysis and altered expression of lipoprotein lipase and hepatic lipase. It leads hyperinsulinemia and affects regulation of lipid metabolism, protein synthesis and modulation of androgen production [21]. It also causes increased secretion of VLDL particles by the liver ends up in elevated triglycerides concentration in serum and it is also contributes more catabolism of HDL particles[22].

Both Homocysteine and lipids are toxic in vascular cells and hepatocytes. Deficiency of methyl group donor and abnormal level of Hcy, both are responsible for deranged phospholipid metabolism which ultimately ends up in impaired VLDL-C secretion from hepatocytes. Hcy plays a role in influencing endoplasmic reticulum (ER) stress as well as also in regulating enzymes those are responsible for HDL-C metabolism [23].

The study was planned to evaluate serum Homocysteine level, lipid profile and an association between Homocysteine and lipid profile in patients with polycystic ovary syndrome.

MATERIALS AND METHODS

The study was conducted in Department of Biochemistry in association with the Department of Obstetrics and Gynecology, Mahatma Gandhi Medical College and Hospital, Jaipur. All the patients fulfilling the inclusion criteria were chosen for the study. Age and sex matched healthy subjects constituted the control group. The study was conducted after seeking approval from the Institutional ethics committee (IEC). Written and informed consent was obtained from all participants prior to enrolment for study.

Total 50 subjects fulfilling the inclusion criteria were taken for study. The control group consisted of fifty (n=50) age matched healthy females.

Patients diagnosed with PCOS based on Rotterdam criteria and ages between 18 to 45 years were included in the study. Pregnant and menopausal women were excluded from the study. Patients with coronary heart disease, hypertension, diabetes mellitus or other endocrine disorders, alcoholics, smokers and patients on vitamin supplementation were also excluded from the study. Blood samples for all subjects (patient and control group) were collected using standard aseptic technique and analyzed for following investigations: serum Homocysteine, Lipid Profile [total cholesterol, HDL, LDL, VLDL and Triglycerides]. Parameters were estimated by fully automated analyzer VITROS 5600.

Statistical Analysis

Result obtained for various parameter were presented as mean+SD among the two groups i.e. PCOS patients (n=50) and control group (n=50). The result of patients group was compared with those of control group by applying student's t-test. To evaluate the association between homocysteine level and component of lipid profile in PCOS patients, Pearson's correlation test was also applied. A p-value of ≤ 0.05 was considered as significant for all statistical tests.

RESULTS AND DISCUSSION

The study population consisted of 100 subjects (50 PCOS patients, 50 controls). Patients were taken from out- patient department of obstetrics and gynecology, Mahatma Gandhi Medical College and Hospital, Jaipur. Patients were selected based on the predefined inclusion and exclusion criteria and after obtaining informed consent. Blood sample was collected and analyzed for estimation of serum Homocysteine, total cholesterol, and triglycerides, HDL, LDL and VLDL. Data of both controls and cases were analyzed statistically. P value <0.05 was found statistically significant.

Mean age in PCOS patients' 26.0±5.03 years and in healthy controls 26.5±3.84 years was not found statistically significant (p value 0.578) as shown in table 1.

The present study showed that serum homocysteine levels were found significantly higher (p value 0.000) in PCOS group than control group with mean value 11.87±5.14 mol/L and 4.9±2.5 mol/L respectively as shown in Table 2. Mohan SK and Priya VV have found that increased Hcy levels and decreased antioxidant capacity may contribute to the increased risk of cardiovascular disease in women with PCOS [24]. Loverro et al. [25] and Badawy et al. [26] have same findings as the present study. Mancici et al., in their study found no significant differences in Hcy levels among PCOS women and healthy controls [27]. Framingham offspring study has demonstrated, that hyperhomocysteinemia is associated with hyperinsulinemia and may partially account for increased risk of cardiovascular disease associated with insulin resistance [28]. Insulin inhibits the hepatic cystathione beta synthase activity, which increases the level of serum Hcy [29]. It has well known role in cardiovascular morbidity and mortality with its atherogenic and prothrombotic properties, molecular mechanisms of Hcy-induced cellular dysfunction include increased inflammatory cytokine expression, induction of oxidative stress, activation of apoptosis and defective methylation [30].

In present study it has been observed that, there is a significant increase in serum total cholesterol in PCOS patients' 313.5±70.42 mg/dL when compared with control group 156.76±32.12 mg/dL (p value 0.000). This study also shows a significant increase in triglyceride level in PCOS patients' 207.18±84.46 mg/dL than control group 104.08±30.2 mg/dL (p value 0.000). Serum HDL level in PCOS patients was less than controls with mean value 35.98±13.81 mg/dL and 45.88±7.47 mg/dL respectively, statistically significant (p value 0.000). PCOS patients had significantly higher mean value of LDL i.e. 236.06±67.99 mg/dL when compared with controls 88.54±7.47 mg/dL (p value 0.000). VLDL was found to be significantly higher in cases than control group with mean value 41.46 ±16.79 mg/dL and 20.84±6.14 mg/dL (p value 0.000) respectively. These results are shown in table 3. Similar results has been found by some studies suggesting that PCOS patients were hyperlipidemic with higher total cholesterol, LDL-C, VLDL-C and triglyceride concentrations and lower HDL-C levels than controls [31].

Hyperinsulinemia and hyperandrogenemia cause adipocytes to undergo increased catecholamine-induced lipolysis and release of free fatty acids into the circulation. Increased free fatty acids in the liver stimulate secretion of VLDL-C, which ultimately leads to hypertriglyceridemia [32]. It has been postulated by Watterau and coworkers that insulin inhibits the expression of the microsomal triglyceride protein, which is responsible for the secretion of VLDL-C. Insulin resistance leads to hepatic overproduction of VLDL and ultimately to hypertriglyceridemia [33]. The increase in triglyceride may be because of the accumulation of triglycerides, which may occur owing to the increased lipogenesis, decreased clearance and reduced oxidation of fatty acids. Insulin resistance also contributes more to catabolism of HDL-C particals and formation of LDL-C. Hyperandrogenism has

been associated with increased hepatic lipase activity and plays a role in the catabolism of HDL-C particles [34].

Further, the association of serum homocysteine levels with components of Lipid profile was evaluated by applying Pearson's correlation. A significant association was observed between serum homocysteine and total cholesterol (r=0.36, p=0.01) and serum homocysteine and LDL-C (r=0.29, p=0.04) represented in table 4, figure 4.1 and 4.2. Association between Hcy and triglyceride (r=0.22, p=0.12), Hcy and HDL-C (r=0.11, p=0.44) and Hcy and VLDL-C (r=0.23, p=0.10) were found to be non significant represented in table 4.

Both Hcy and lipids are toxic for vascular cells and hepatocytes. Hyperhomocysteinemia (HHcy) and hypercholesterolemia are linked to the development of atherothrombotic diseases. The risk associated with HHcy and hypercholesterolemia is greater than that associated with only one of these risk factors. Mechanism by which cells those are sensitive to elevated intracellular levels of Hcy results in ER-stress that leads to activation and dysregulation of endogenous sterol response pathway. The sterol regulatory element- binding protein (SREBP-1) is an ER-membrane bound transcription factor that activates genes encoding key enzyme in the cholesterol biosynthesis and uptake pathways. Hcy has been found to enhance the expression of SREBPs and in this way it can enhance intracellular accumulation of cholesterol. Hcy cause protein misfolding in the ER and activates the unfolded protein response (UPR) thus causing increased expression of ER stress-response genes. An association between UPR activation and lipid biosynthesis has been demonstrated in human fibroblasts [23]. Qujeq D, Omran TS, Hosini L were found significantly positive correlation between Hcy and LDL-C in myocardial infarction patients [35]. Oxidative stress is one mechanism by which Hcy might affect lipoprotein particles and thereby damage endothelial cells. Hcy can enhance hydroxyl radical generation and formation of oxidized and homocysteinylated- LDL. The modified forms of LDL are more toxic than the native LDL and are readily taken up by macrophages thus facilitating the initiation and progression of the inflammatory response in the endothelial lesions [23].

Table 1: Comparison Of Age (years) Between Control And PCOS Group.
NS= Non Significant

Groups	AGE (years)	t-Value	P-Value
Control (n=50)	26.5±3.84	-0.559	0.578 (NS)
PCOS (n=50)	26.0±5.03		

Table 2: Comparison Of Homocysteine (µmol/L) Between Control And PCOS Group.

Groups	Homocysteine (µmol/L)	t-Value	P-Value
Control (n=50)	4.90±2.5	8.623	0.000
PCOS (n=50)	11.87±5.14		

Table 3: Comparison Of Lipid Profile Between Control And PCOS Group.

Parameters	Control group	PCOS patients	t-value	p-value
Total Cholesterol (mg/dL)	156±32.12	313.5±70.42	14.319	0.000
Triglycerides (mg/dL)	104.08±30.2	207.18±84.46	8.128	0.000
HDL(mg/dL)	45.88±7.47	35.98±13.81	-4.459	0.000
LDL(mg/dL)	88.54±28.44	236.06±68.68	14.033	0.000
VLDL (mg/dL)	20.84±6.14	41.46±16.96	8.084	0.000

Table 4: Correlation Between Serum Homocysteine Levels With Lipid Profile In PCOS Patients.

Test	Correlation Coefficient (r)	P value
Hcy v/s Total Cholesterol	0.36	0.01
Hcy v/s Triglyceride	0.22	NS
Hcy v/s HDL	0.11	NS
Hcy v/s LDL	0.29	0.04
Hcy v/s VLDL	0.23	NS

R and P- value as obtained on applying Pearson's correlation

CONCLUSION

The study shows increased level of Hcy, total cholesterol, TG, LDL and VLDL and decreased level of HDL in patients with PCOS when compared with control group. A significant association was also observed between Hcy and Total cholesterol and Hcy and LDL in PCOS patients. Increased Hcy level and dislipidemia are major risk factors for cardiovascular diseases and type II diabetes mellitus. In PCOS patients' dislipidemia and HHcy can be used as sensitive indicator for assessment of CVD. Estimation of serum Hcy and lipid profile is therefore, recommended as a part of routine screening and follow up of PCOS patients. The clinical and epidemical data concerning correlation between Hcy and TC, LDL-C are very limited, indicating that more studies are needed.

Vitamin B6, vitamin B12 and folic acid are major dietary factors those affects Hcy level. The study therefore recommends further research on the association of homocysteine levels with concentration of vitamin B12 and folic acid in patients with polycystic ovary syndrome.

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