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IL-6 -G174C PROMOTER GENE POLYMORPHISM, METABOLIC RISK MARKERS AND HYPERANDROGENEMIA IN POLYCYSTIC OVARIAN SYNDROME

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ABSTRACT

The present study was aimed to assess the IL-6 G174C gene polymorphism, metabolic risk markers, hyperandrogenemia and Insulin Resistance in PCOS women. Total 439 women were enrolled. Circulating IL-6, Insulin, lipid profile, hyperandrogenemic parameters were measured in the groups and subgroups, insulin resistance was calculated and IL-6- G174C genotyping was done. The observation shows significantly high frequency distribution of mutant genotype CC+GC (p=<0.001; OR=2.76, 95%CI=1.69-4.49) and mutant C allele (p=<0.001, OR=1.75, 95%CI=1.34-2.28) in PCOS women compared to non-PCOS group and in metabolic subgroups compared to non metabolic subgroups. In conclusion, the mutant C allele and mutant genotype of IL-6 promoter gene polymorphism were significantly associated with PCOS, high circulatory IL-6 and metabolic risk markers irrespective of presence or absence of PCOS. Women having mutant genotype of IL-6 gene and high circulatory IL-6, central obesity, Insulin resistance and dyslipidemia are more prone for development of cardio metabolic diseases.

KEYWORDS: **Metabolic risk markers, Polycystic ovary syndrome, Insulin resistance, IL-6 promoter gene polymorphism, Circulating IL-6.**

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is a common heterogeneous disorder characterized by hyperandrogenism, chronic anovulation and infertility. It affects 5-10% of women of reproductive age and is associated with an increased prevalence of generalized and abdominal obesity [1]. In recent years, several studies have demonstrated high risk for impaired glucose tolerance and type II Diabetes Mellitus in PCOS [2] It is still not very clear, whether this increase in risk is related to endocrine abnormalities such as hyperandrogenemia associated with PCOS or whether it is a consequence of anthropometrical and metabolic abnormalities frequently observed in PCOS? Hyperinsulinemia insulin resistance is intrinsic to PCOS [3] affects obese and lean women with PCOS. 50-60% of women with PCOS are obese and they additionally possess a form of insulin resistance that is directly associated to obesity [4.] Obesity, insulin resistance and dyslipidemia are emerging risk factors for metabolic syndrome and cardiovascular diseases. In addition to obesity and insulin resistance, some study suggests that hormonal and metabolic factors in young PCOS subjects are strongly associated with insulin resistance, and may concur to determine a condition of low-grade inflammation eliciting an overproduction of IL-6 monocytes in IR PCOS subjects [5.] PCOS has been linked to elevated inflammatory markers of atherosclerosis disease and genes coding for inflammatory cytokines are therefore considered as candidates for predisposition to risk of coronary heart disease and has been identified as target genes for PCOS [6]. Few studies have explored the predictive value of inflammatory markers on cardiovascular disease and other have considered multiple markers, such as interleukin-6 (IL-6), C-reactive protein (CRP), and tumor necrosis factor-α (TNF-α) [7]. Interleukin-6 has a cytokine, endocrine, paracrine and autocrine actions and are believed to affect the process of fertilization and implantation [9]. Increase circulating IL-6 is found to be present in some of the women with PCOS [10]. A study on mice shows that IL-6 alters vital steps in follicular maturation, ultimately contributing to ovarian dysfunction [11]. Thus, IL-6 may provide a link between anthropometric and metabolic alteration and hyperandrogenemia in PCOS. A common G/C single nucleotide polymorphism of the IL-6 promoter at position 174 has been found to influence the transcription rate of this multifunctional cytokine [12] and is supposed to be involved in the regulation of androgen levels [13], IL-6 promoter region polymorphism may be related to occurrence and metabolic abnormalities seen in PCOS in the Turkish population [14], insulin sensitivity and energy expenditure [15]. It has been proposed that subjects with mutant 'C' allele of IL-6 have an increased risk of metabolic disorder associated with PCOS, such as insulin resistance, Type-II diabetes, and lipid abnormalities [16]We have correlated IL-6 -G174C promoter gene polymorphism with circulating IL-6, insulin resistance and metabolic risk markers in PCOS. A second objective was to determine if correlation exists between IL-6 -G174C promoter gene polymorphism and hyperandrogenemic features of PCOS women.

MATERIALS AND METHODS

In this case control study total 439 female subjects (Age 25±10 yr, range 15-35 yr) from Department of Obstetric & Gynae, Queen Mary Hospital, King George's Medical University, Lucknow, and Ajanta Hospital (Infertility centre), Lucknow were enrolled for the study. 223 PCOS women diagnosed on the basis of revised criteria for PCOS following the 2003 Rotterdam consensus [17] were taken as study group, which were further divided into subgroups as women with metabolic syndrome; n=133 (Group 1) and women without metabolic syndrome ; n=90 (Group 2) as per NCEP ATP-III criteria and 216 non-PCOS women were enrolled as a control group which were further divided into subgroups as women with metabolic syndrome; n=110 (Group 3)

and women without metabolic syndrome; n=106 (Group 4) as per NCEP ATP-III. The inclusion criteria's for non-PCOS women were absence of PCOS on transvaginal ultrasonography and absence of biochemical and clinical features of hyperandrogenemia, other inclusion criteria for study and control group were teetotaler, non-alcoholic, non-diabetic, without any kind of cardiac, respiratory, inflammatory, endocrinal and metabolic disease women. Pregnant, lactating and women with any kind of gynecological or obstetrical problems and on medication including hormone replacement therapy, with any viral, bacterial, allergic and inflammatory diseases were excluded from the study. Informed consent was obtained from all the subjects for participation in the study. Ethical clearance from the Institutional ethics committee of King George's Medical University, Lucknow was obtained and "we certify that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during this study".All the women were studied on the 10th days after onset of menstruation in case of normal menstruation and Oligomenorrhea. Information regarding medical, personal, family, dietary and menstrual history was obtained in the form of questionnaire. Clinical and anthropometric variables, including a modified hirsutism score [18], body mass index, and waist-hip ratio, were determined in all the subjects. WHR was measured as abdominal circumference at Umbilicus/Hip circumference at greater trochantor level.

BLOOD COLLECTION, DNA EXTRACTION AND BIOCHEMICAL ANALYSIS

Total 6.0 ml venous blood sample for measuring serum biochemical and lipid profiles, were obtained in the morning after fasting of 12 hours from each subject on the 10th days after onset of menstruation, 2.0 ml blood was collected in EDTA vial. DNA was extracted from blood by using a commercial kit (Qiagen, USA). From the rest 4.0 ml of the blood, 2 ml serum and 1 ml plasma was separated out. Estimation of plasma Glucose was done by GOD-POD method (Randox Laboratories Ltd., Antrim, UK). Serum Insulin was estimated by Immuno-radiometric assay method (Immunotech Radiova, Prague), Serum IL-6 was measured by ELISA method (Quantikine IL-6, R&D system Oxford, UK), DHEAS and total testosterone was assayed by a Competitive immunoenzymatic colorimetric method purchased from Diametra (Italy) with a lower sensitivity of 0.045µg/ml and 0.07 ng/ml respectively and intra- and interassay coefficients of variation of 4.8%, 5.4% and 8.9% , 15% respectively, whereas sex hormone binding globulin (SHBG) were assayed by direct immunoenzymatic colorimetric method respectively purchased from Diametra (Italy). Lipid profile was estimated by enzymatic method (Randox Laboratories Ltd., Antrim, UK). Insulin resistance was also calculated by homeostatic model assessment index (HOMA Index) [19] using the equation: HOMA Index = [fasting Insulin (µU/l) x fasting glucose (mmol/l)/ 22.5] If subjects had no clinical and biological criteria of Insulin resistance, a laboratory diagnosis of IR was made on the basis of HOMA Index ≥ 3.6 [20].

IL-6 -G174C GENOTYPING

The genotyping protocol for the detection of the IL-6 -G174C polymorphism was analyzed as per Fishman et al [12]. To improve the genotyping quality and validation, all mutant and heterozygous samples were re-genotyped and results were noted only for those samples which were reproducible and with no discrepancy.

STATISTICAL ANALYSIS

Statistical analysis was carried out using the STATISTICA version 6.0. Quantitative variables are presented as the mean ± standard deviation. Comparisons of continuous data between two independent groups were done by Student unpaired t-test while categorical data was analyzed by chi-square (χ^2) test or Fisher's exact test, as appropriate and categorical data was also analyzed by Cochran Armitage trend test using XLSTAT version 12 , A two-tailed (α=2) welch corrected probability $p<0.05$ was considered statistically significant.

Allelic and genotypic frequencies were estimated by genotype count. The Hardy-Weinberg equilibrium assumptions were assessed by comparing the observed and expected numbers of genotypes. Logistic regression was done Minitab version 16. The association between genotypes and clinical characteristics was expressed as odds ratio (OR) with 95% confidence interval (95% CI). The power analysis was performed for sample size taking 95% Confidence Interval (CI), 5% expected error and 8% prevalence.

RESULTS

Present study is based upon 223 PCOS and 216 non PCOS women (Age 25±10 yr, range 15-35 yr). Statistically significant high values for Glucose (p=0.004), WHR (p=0.019), SBP (p=0.009), DBP (p=0.047), TC (p=<0.001), TG (p=0.001), Insulin (p=0.008), HOMA Index (HOMA-IR) (p=0.004), circulating IL-6 level (p=0.009) and lower HDL (p=<0.001) were observed in PCOS women compared to non-PCOS women (Table 1). Furthermore, PCOS women with metabolic syndrome (Group- 1) had statistically significant high values for Glucose (p=<0.001), BMI (p=<0.001), WHR (p=<0.001), SBP (p=<0.001), DBP (p=<0.001), Total Cholesterol (p=<0.001), TG (p=<0.001), Insulin (p=<0.001), HOMA Index (IR) (p=<0.001), IL-6 (p=0.008) and lower HDL (p=<0.001) compared to PCOS women without metabolic syndrome (Group - 2). Similarly, statistically significant high values were observed in non-PCOS women with metabolic syndrome (Group- 3) for Glucose (p=<0.001), BMI (p=<0.001), WHR (p=<0.001), SBP (p=<0.001), DBP (p=<0.001), Total Cholesterol (p=<0.001), TG (p=<0.001), Insulin (p=<0.001), HOMA Index (IR) (p=<0.001) and IL-6 (p=0.03) compared to non-PCOS women without metabolic syndrome (Group- 4). On comparison of PCOS women with metabolic syndrome (Group- 1) with non PCOS women with metabolic syndrome (Group- 3), significant high value were found for Glucose (p=0.034), BMI (p=0.002), TC (p=<0.001), IL-6 (p=0.011) and lower HDL (p=<0.001), however; on comparison of PCOS women without metabolic syndrome (Group-2) with non PCOS women without metabolic syndrome (Group- 4), significant high value were observed only for Glucose (p=0.007) and TG (p=0.021) (Table II)On accessing the hyperandrogenemic clinical and biochemical parameters it was observed ; that PCOS women with metabolic syndrome (Group-1) have significant higher levels of hirsutism (p=0.029) and total testosterone (p=<0.001) and lower level of SHBG (p=<0.001) and DHEA-S (p=0.021) compared to PCOS women without metabolic syndrome (Group- 2) Similarly, non PCOS women with metabolic syndrome (Group- 3) also have significantly higher levels of hirsutism (p=<0.001), total testosterone (p=0.002) compared to non-PCOS women without metabolic syndrome (Group- 4). (Table II) These observation signifying that total testosterone and SHBG also have link with metabolic syndrome independent of presence or absence of PCOS.Unadjusted and adjusted odd ratio were calculated in PCOS and non PCOS women, unadjusted odd ratio was significant (p=<0.05) for all variables while adjusted odd ratio was significant for Glucose (p=0.045), BMI (p=<0.001), TC (p=0.019), Insulin resistance (p=0.04) (Table III). Similarly in PCOS women with and without metabolic syndrome, unadjusted odd ratio was significant (p=<0.05) for all the variable while adjusted odd ratio was significant for SBP (p=0.004), TC (p=0.004), TG (p=0.002), Insulin (p=0.048) and IL-6 (p=0.041) however in non PCOS women with and without metabolic syndrome, unadjusted odd ratio was significant (p=<0.05) for all variable while adjusted odd ratio was significant only for SBP (p=0.002) and TG (p=0.009) (Table IV)Significantly high frequency distributions of CC (Homozygous mutant) genotype of IL-6 -G174C gene polymorphism were observed in PCOS and with metabolic syndrome sub-group of PCOS as well as non PCOS women. While significantly high frequency distributions of mutant C allele of IL-6 -G174C gene were observed in PCOS and with metabolic syndrome sub-group of non PCOS women. (Table V)We also made

an attempt to correlate metabolic risk markers with IL-6 G/C gene polymorphism on the basis of presence of mutant C allele and genotype in PCOS and Non-PCOS women. It was observed that homozygous mutant CC as well as heterozygous GC is more significantly associated with WHR (p=0.003), TC (p=<0.001), IR (p=<0.001) and IL-6 (p=0.004) in PCOS women and with Glucose (p=0.007), BMI (p=0.005), SBP (p=0.041), DBP (p=0.039) and TC (p=0.004), IR (p=<0.001) and IL-6 (p=<0.001) in non PCOS women. (Table VI) Similarly homozygous mutant CC as well as heterozygous GC is more significantly associated with WHR (p=0.013), IR (p=0.002) and IL-6 (p=0.022) in PCOS women with metabolic syndrome and with Glucose (p=0.016), WHR(p=0.045), TC (p=0.030), IR (p=0.014) and IL-6 (p=0.006) in non PCOS women with metabolic syndrome. (Table VII) On analyzing frequency association of mutant C allele and genotype of IL-6 -G174C gene polymorphism with clinical and biochemical signs of hyperandrogenemia in PCOS women. We observed that homozygous mutant CC as well as heterozygous GC is more significantly associated with hirsutism (p=0.023), total testosterone (p=0.008) and DHEA-S (p=<0.001) in PCOS women (Table-VIII), and more significantly associated with total testosterone (p=0.011) and DHEA-S (p=0.001) in PCOS women with metabolic syndrome while there is no difference were observed in non-PCOS women with metabolic syndrome (Table-IX)

Table I
Metabolic Risk markers, circulatory IL-6 and Insulin resistance in PCOS and non-PCOS women

Metabolic risk markers	PCOS women (n=223)	Non-PCOS women (n=216)	p-value
Glucose (mg/dl)	104.51 ± 20.18	97.97 ± 18.05	0.004*
BMI (Kg/m^2)	26.46 ± 10.44	26.02 ± 4.55	0.568
WHR	0.87 ± 0.04	0.86 ± 0.05	0.019*
SBP (mmHg)	127.04 ± 13.06	123.90 ± 12.30	0.009*
DBP (mmHg)	84.88 ± 8.58	83.33 ± 7.68	0.047*
TC (mg/dl)	178.83 ± 40.74	158.60 ± 34.80	<0.001
HDL (mg/dl)	40.69 ± 5.52	43.13 ± 4.52	<0.001
TG (mg/dl)	134.92 ± 38.66	123.83 ± 35.40	0.001*
Insulin	12.21 ± 6.78	10.34 ± 7.95	0.008*
IR	3.26 ± 2.04	2.65 ± 2.41	0.004*
IL-6 (pg/ml)	6.08 ± 4.18	4.85 ± 3.43	0.009*

Data are presented as mean ±SD, A value of p<0.05 was considered statistically significant
BMI: body mass index; WHR: waist to hip ratio; SBP: systolic blood pressure; DBP: diastolic blood pressure; TC: total cholesterol; HDL: high density lipoprotein; TG: triglyceride; IR: insulin resistance.

Table II

Metabolic Risk markers and hyperandrogenemic features in PCOS, non-PCOS group and subgroups (With Mets & without Mets) women

Metabolic risk Markers and Hyperandrogenemic Features	PCOS MetS (n=133) Group 1	PCOS wMetS (n=90) Group 2	Non-PCOS MetS (n =110) Group 3	Non-PCOS wMets (n= 106) Group 4	p- value Group 1 vs. Group 2	p- value Group 3 vs. Group 4	p- value Group 1 vs. Group 3	p- value Group 2 vs. Group 4
Glucose (mg/dl)	112.79 ± 21.22	92.30 ± 9.74	107.23 ± 19.06	88.35 ± 10.34	<0.001*	<0.001*	0.034*	0.007*
BMI (Kg/m^2)	30.02 ± 3.71	23.84 ± 4.00	28.39 ± 4.71	23.55 ± 2.72	<0.001*	<0.001*	0.002*	0.549
WHR	0.89 ± 0.03	0.83 ± 0.05	0.88 ± 0.05	0.84 ± 0.05	<0.001*	<0.001*	0.055	0.165
SBP (mmHg)	133.23 ± 12.51	117.91 ± 7.21	130.26 ± 11.93	117.30 ± 8.71	<0.001*	<0.001*	0.0612	0.598
DBP (mmHg)	88.72 ± 8.01	79.2 ± 5.79	86.87 ± 7.43	79.66 ± 6.06	<0.001*	<0.001*	0.065	0.589
TC (mg/dl)	200.01 ± 34.95	147.54 ± 25.81	174.56 ± 34.51	142.04 ± 26.47	<0.001*	<0.001*	<0.001*	0.144
HDL (mg/dl)	38.94 ± 4.01	43.28 ± 6.39	42.11 ± 4.35	42.23 ± 4.77	<0.001*	0.847	<0.001*	0.190
TG (mg/dl)	136.36 ± 42.92	108.01 ± 22.60	145.91 ± 32.84	100.92 ± 20.16	<0.001*	<0.001*	0.056	0.0213
Insulin	14.15 ± 6.64	9.35 ± 5.93	12.47 ± 9.50	8.13 ± 5.10	<0.001*	<0.001*	0.107	0.123
IR	4.00 ± 2.06	2.16 ± 1.42	3.46 ± 2.94	1.82 ± 1.26	<0.001*	<0.001*	0.94	0.077
IL-6 (pg/ml)	6.84 ± 4.79	4.95 ± 2.71	5.34 ± 4.24	4.33 ± 2.21	0.008*	0.030*	0.011*	0.079
Hirsutism (FG)	14.14 ± 7.80	11.98 ± 6.27	2.03 ± 1.56	1.38 ± 1.45	0.029*	<0.001*	<0.001*	<0.001*
Acne (%)	43 (32.33%)	18(20%)	13 (11.82%)	08 (7.55%)	0.061	0.406	0.003*	0.019*
Alopecia(%)	15(11.28%)	08(8.89%)	06 (5.45%)	01 (0.94%)	0.726	0.137	0.168	0.021*
Oligomenorrhea(%)	51(38.34%)	60(66.67%)	20 (18.18%)	18 (16.98%)	<0.001*	0.958	0.001*	<0.001*
Amenorrhea (%)	38(28.57%)	19(21.11%)	0	0	0.272	-	<0.001*	<0.001*
Tot. Testosterone(ng/ml)	0.74 ± 0.48	0.55 ± 0.24	0.44 ± 0.21	0.36 ± 0.17	<0.001*	0.002*	<0.001*	<0.001*
SHBG (nmol/l)	30.24 ± 11.18	40.50 ± 16.83	43.60 ± 8.04	48.54 ± 30.06	<0.001*	0.097	<0.001*	<0.001*
DHEA-S (µg/ml)	2.67 ± 1.31	2.28 ± 1.06	1.52 ± 0.74	1.58 ± 0.72	0.021*	0.540	<0.001*	<0.001*

Data are presented as mean ±SD, A value of p<0.05 was considered statistically significant
MetS; Metabolic Syndrome, wMetS; Without Metabolic Syndrome

Table III

Unadjusted and Adjusted Odd- ratio for metabolic risks markers, circulatory IL-6 and Insulin resistance among PCOS and Non PCOS women

S. No	Metabolic Risk Markers	Unadjusted Odd- ratio	(95 % CI)	P value	Adjusted Odd ratio	(95 % CI)	P value
1.	Glucose	0.98	(0.97-0.99)	0.001*	0.98	(0.95-1)	0.045*
2.	BMI	0.83	(0.80-0.87)	<0.001*	0.83	(0.79-0.88)	<.001*
3.	SBP	0.98	(0.97-1)	0.011*	0.99	(0.97-1.2)	0.697
4.	DBP	0.98	(0.95-1)	0.049*	1.03	(0.98-1.07)	0.203
5.	TC	0.99	(0.98-0.99)	<0.001*	0.99	(0.98-1)	0.019*
6.	TG	1.06	(1.02-1.10)	0.003*	1.01	(0.96-1.05)	0.731
7.	Insulin	0.96	(0.94-0.99)	0.01*	0.85	(0.71-1.01)	0.062
8.	IR	0.88	(0.81-0.96)	0.006*	1.93	(1.02-3.67)	0.04*
9.	IL-6	0.91	(0.81-0.96)	0.001*	0.99	(0.93-1.06)	0.854

A value of p<0.05 was considered statistically significant

Table IV

Unadjusted and Adjusted Odd- ratio for metabolic risks markers, circulatory IL-6 and Insulin resistance among PCOS, non PCOS group and subgroups (With Mets & without Mets) women

		PCOS with and without metabolic syndrome						Non-PCOS with and without metabolic syndrome					
S. No.	Metabolic Risk Mrakers	Unadjusted Odd-Ratio	95% CI	p Value	Adjusted Odd-Ratio	95% CI	p Value	Unadjusted Odd-Ratio	95% CI	p Value	Adjusted Odd-Ratio	95% CI	p Value
1.	Glucose	1.09	1.06- 1.12	<0.001*	1.04	0.87- 1.25	0.672	1.09	1.06- 1.12	<0.001*	1.03	0.86- 1.23	0.757
2.	BMI	1.31	1.21- 1.41	<0.001*	1.22	0.94- 1.57	0.132	1.43	1.29- 1.58	<0.001*	1.23	0.97- 1.57	0.093
3.	SBP	1.21	1.15-1.27	<0.001*	1.36	1.10- 1.68	0.004*	1.14	1.10- 1.19	<0.001*	1.40	1.13- 1.73	0.002*
4.	DBP	1.28	1.20-1.38	<0.001*	0.94	0.76- 1.17	0.576	1.20	1.13- 1.27	<0.001*	0.90	0.73- 1.11	0.338
5.	TC	1.06	1.05-1.08	<0.001*	1.01	0.97- 1.04	0.004*	1.04	1.03- 1.05	<0.001*	1.01	0.98- 1.05	0.484
6.	TG	1.06	1.05- 1.08	<0.001*	1.12	1.04- 1.20	0.002*	1.06	1.05- 1.08	<0.001*	1.11	1.04- 1.18	0.001*
7.	Insulin	1.15	1.09-1.21	<0.001*	1.28	0.97- 1.16	0.048*	1.10	1.05- 1.15	<0.001*	0.21	0.03- 1.27	0.089
8.	IR	1.94	1.57-2.41	<0.001*	2.53	0.44- 4.35	0.081	1.59	1.31- 1.93	<0.001*	4.47	0.33- 5.87	0.097
9.	IL-6	1.71	1.07-1.28	0.001*	1.04	0.62- 1.42	0.041*	1.10	1.01- 1.19	0.035*	0.98	0.66- 1.45	0.916

A value of p<0.05 was considered statistically significant

Table V

Distribution frequency of IL6 –G174C gene polymorphism in PCOS, non-PCOS group and subgroups (with Mets & without Mets) women

IL-6 174G/C polymorphism	Non-PCOS With Mets (n= 110)	Non- PCOS Without Mets (n =106)	p- value OR (95%CI)	PCOS With Mets (n=133)	PCOS Without Mets (n=90)	p- value OR (95%CI)	PCOS n=223	Non- PCOS n= 216	p- value OR (95%CI)
Genotype									
GG	23	40		10	19		29	63	
GC	65	48	0.010*	84	43	0.005*	127	113	<0.001*
CC	22	18	2.29(1.25-4.20)	39	28	3.29(1.45- 7.47)	67	40	2.76(1.69-4.49)
Allele		**Cochran Armitage trend test (P = 0.03)**			**Cochran Armitage trend test (P = 0.172)**			**Cochran Armitage trend test (P =< 0.001)**	
G	111	128		104	81	0.252	185	239	<0.001*
C	109	84	0.048* 1.49(1.02-2.19)	162	99	1.27(0.86-1.87)	261	193	1.75(1.34-2.28)

A value of p<0.05 was considered statistically significant

Table VI

Comparison between frequency distribution of wild (GG) and Mutant genotype (GC+CC) among PCOS and non-PCOS women.

Metabolic risk Markers and Hyperandrogenemic features	PCOS (n=223)		P value	Non-PCOS (n=216)		p- value
	GG (n= 29)	GC+CC (n= 194)		GG (n=63)	GC+CC (n= 153)	
Glucose (mg/dl)	101.54 ± 17.72	104.96 ± 20.52	0.395	92.88 ± 14.95	100.06 ± 18.84	0.007*
BMI (Kg/m^2)	24.15 ± 4.67	26.81 ± 11.02	0.202	24.68 ± 4.32	26.57 ± 4.55	0.005*
WHR	0.84 ± 0.06	0.87 ± 0.05	0.003*	0.82 ± 0.06	0.86 ± 0.04	<0.001*
SBP (mmHg)	126.34 ± 15.23	127.15 ± 12.75	0.756	121.24 ± 11.68	125.00 ± 12.43	0.041*
DBP (mmHg)	84.06 ± 8.44	85.00 ± 8.61	0.583	81.65 ± 7.74	84.02 ± 7.58	0.039*
TC (mg/dl)	161.16 ± 39.72	189.99 ± 35.86	<0.001*	148.16 ± 34.60	162.90 ± 34.08	0.004*
HDL (mg/dl)	40.74 ± 6.54	40.69 ± 5.38	0.964	42.68 ± 4.28	41.90 ± 4.62	0.250
TG (mg/dl)	123.33 ± 47.53	125.16 ± 37.29	0.812	119.28 ± 37.14	125.71 ± 34.62	0.226
Insulin	10.28 ± 6.17	12.51 ± 6.83	0.098	9.88 ± 6.91	10.54 ± 7.02	0.528
IR	2.62 ± 1.79	4.35 ± 2.06	<0.001*	2.38 ± 1.43	3.79 ± 1.39	<0.001*
IL-6 (pg/ml)	5.39 ± 4.18	7.68 ± 4.03	0.004*	5.03 ± 3.42	6.78 ± 2.41	<0.001*

Data are presented as mean ±SD, A value of p<0.05 was considered statistically significant

Table VII
Comparison between frequency distribution of wild (GG) and Mutant genotype (GC+CC) among PCOS and non-PCOS with MetS.

Metabolic risk Markers and Hyperandrogenemic features	PCOS with MetS (n=133)		p- value	Non-PCOS with MetS (n=110)		p- value
	GG (n= 15)	GC+CC (n= 118)		GG (n=23)	GC+CC (n= 87)	
Glucose (mg/dl)	110.56 ± 19.92	113.07 ±21.44	0.667	98.11 ± 16.51	108.98 ± 19.63	0.016*
BMI (Kg/m^2)	27.79 ± 2.29	28.49 ±2.75	0.347	27.11 ± 5.42	28.74 ± 4.47	0.140
WHR	0.88 ± 0.02	0.90 ±0.03	0.013*	0.87 ± 0.05	0.89 ±0 .04	0.045*
SBP (mmHg)	132.87 ± 17.51	133.14 ± 11.82	0.937	130.78 ± 10.10	132.13 ± 12.42	0.631
DBP (mmHg)	87.87 ± 9.69	88.83 ± 7.82	0.663	86.69 ± 7.23	87.91 ± 7.53	0.487
TC (mg/dl)	197.24 ± 36.54	200.37 ± 34.89	0.745	168.41 ± 37.38	186.18 ± 33.75	0.030*
HDL (mg/dl)	40.03 ± 4.56	39.05 ± 3.94	0.373	40.74 ± 3.73	39.77 ± 3.73	0.269
TG (mg/dl)	145.55 ± 55.39	148.19 ± 41.22	0.823	150.45 ± 39.16	144.71 ± 31.11	0.458
Insulin	12.20 ± 7.74	14.40 ± 6.49	0.228	11.15 ± 9.36	12.02 ± 8.62	0.673
IR	3.33 ± 2.20	5.08 ± 2.04	0.002*	3.19 ± 2.45	4.79 ± 2.81	0.014*
IL-6 (pg/ml)	5.76 ± 4.29	8.27 ± 3.91	0.022*	5.18 ± 4.99	7.97 ± 4.03	0.006*

Data are presented as mean ±SD, A value of p<0.05 was considered statistically significant

Table VIII
Clinical and Biochemical signs of hyperandrogenemia in PCOS women on the basis of presence of mutant C allele of IL6 -G174C gene polymorphism

Variables	GG n = 29 (%)	GC+CC n = 194(%)	p- value
Hirsutism (FG)	11.00±6.69	14.31±7.39	0.023*
Acne (%)	6	55	0.504
Alopecia (%)	4	19	0.513
Oligomenorrhea (%)	10 (29)	101 (58)	0.110
Amenorrhea (%)	7 (25)	50 (26.5)	1.00
Total Testosterone (ng/ml)	0.62±0.23	0.76±0.27	0.008*
SHBG (nmol/l)	37.20±17.30	33.96±14.14	0.265
DHEA-S (µg/ml)	2.44±1.18	3.53±1.24	<0.001*

Data are presented as mean ±SD, A value of p<0.05 was considered statistically significant

Table IX.
Clinical and Biochemical signs of hyperandrogenemia in PCOS women and non PCOS women with MetS on the basis of presence of mutant C allele of IL6 -G174C gene polymorphism

Variable	PCOS With MetS (n= 133)		P value	Non PCOS With MetS (n= 110)		P Value
	GG (n= 15)	GC+CC (n= 118)		GG (n= 23)	GC+ CC (n= 87)	
Hirsutism (FG)	12.52 ± 8.13	15.22 ± 7.80	0.221	2.01 ± 1.45	2.04 ± 1.59	0.938
Acne (%)	3	37	0.551	2	11	1.00
Alopecia (%)	2	13	0.677	1	5	1.00
Oligomenorrhea (%)	8	73	0.580	3	17	0.559
Amenorrhea (%)	5	39	1.00	0	0	-
Total Testosterone (ng/ml)	0.64 ± 0.33	0.84 ± 0.28	0.011*	0.42 ± 0.15	0.44 ± 0.22	0.707
SHBG (nmol/l)	31.97 ± 11.64	30.01 ± 11.09	0.524	46.60 ± 17.04	42.82 ± 18.31	0.373
DHEA-S (µg/ml)	2.71 ± 1.44	3.86 ± 1.31	0.001*	1.55 ± 0.79	1.51 ± 0.73	0.792

Data are presented as mean ±SD, A value of p<0.05 was considered statistically significant

DISCUSSION

PCOS is the most common endocrine disorder in women with an enigmatic pathophysiological and molecular basis. One third of the women with PCOS have glucose intolerance and multiple risk factors for cardiovascular disease, including central obesity. Interleukin-6, a cytokine that is mainly derived from adipose tissue, recently implicated as an important link in the development of metabolic and cardiovascular diseases [21]. In the present study, we have examined the association between IL-6 -G174C promoter gene polymorphism, circulating IL-6, Insulin resistance and metabolic risk markers and hyperandrogenemic features in PCOS women. Our study revealed that women with PCOS had high mean values of circulatory IL-6 as compared to non-PCOS women and this increase in serum IL-6 was more associated with metabolic syndrome irrespective of presence or absence of PCOS concordant with previous studies [22,23] and because the rate of transcription is an important regulator of IL-6 expression, genetic variation within regulatory regions of the IL-6 gene could contribute to altered expression of the IL-6 as evident by results of present study showing that the women with mutant genotype of IL-6 -G174C gene polymorphism had significantly higher levels of circulating IL-6 in PCOS women as well as in metabolic subgroups of PCOS and non PCOS women. Furthermore, women with mutant genotype of IL-6 -G174C gene polymorphism shows a significant trend towards central Obesity WHR [24] total cholesterol, Insulin resistance and circulatory IL-6. This could be because the IL-6 gene polymorphism increased the expression of the IL-6 which is the inflammatory cytokine and inflammation is one of the attributing factors for obesity which may lead to hyperinsulinemia and insulin resistance. Our observations are concordant with other studies [25,26] which reported elevated serum levels of inflammatory markers such as TNF-α, IL-6 and hs-CRP in women with PCOS. In addition, changes in serum concentrations of IL-6 paralleled with the changes of abdominal fat mass in PCOS women. These were correlated to obesity as well as to insulin resistance; both of which are common findings of PCOS [27]. Our results revealed that PCOS women with mutant genotype (CC+ GC) were more likely to have high circulatory IL-6, WHR i.e. central obesity, TC and Insulin resistance, similarly the non PCOS women also have high circulatory IL-6, WHR i.e central obesity, BMI, Glucose, SBP, DBP, TC, and Insulin resistance. Adipose tissue accounts up to 30% of total circulatory IL-6 concentration in healthy subjects [28]. Waist to Hip ratio (WHR) is a good indicator of central obesity and has been shown to correlate to type 2 diabetes [29] and metabolic syndrome. Thus, metabolic risk factors like central obesity (WHR), hypercholesterolemia and insulin resistance were more strongly correlated to the presence of mutant genotype of IL-6 174 promoter gene as compared to wild genotype in PCOS and non PCOS women in present study. However, there are fewer evidences suggesting the effect of IL-6 on insulin action. One recent study reports a reduction in adipocytes IRS-1 (insulin receptor substrate-1), GLUT4 expression and tyrosine phosphorylation in response to IL-6 treatment [30] , another study also shows that IL-6 may be an early low-grade chronic inflammatory marker among PCOS patients with IRS-2 (insulin receptor substrate-2) polymorphism in Taiwanese population [31]., In our study, PCOS women showed higher value for Glucose, WHR, SBP, DBP, TC, Insulin, HOMA Index (HOMA-IR) and circulatory IL-6 compared to non-PCOS women. This is in concordant with other studies which have also shown that women with PCOS have increased serum concentrations of CVD risk markers [32] more insulin resistant [33] and had a dyslipidemia associated with insulin-resistant states [34]. This effect of PCOS was explained by a more central fat accumulation [35] or central obesity. The C-174G polymorphism has also been associated in some but not in all studies with increased hyperandrogenism through obesity [36,37,38] as central obesity is one of the attributing factor for hyperandrogenemia [39]. Recently Matthias et al 2004 [40] found that the heterozygous GC genotype, was associated

with lower androstendione and a tendency to lower testosterone levels rather than to hyperandrogenemia but another study by Villuendas et al 2002 [41] involving 85 patients and 25 healthy women from Spain shows that common G alleles of the -597A and C allele of -174G/C IL-6 gene polymorphisms, which are in linkage disequilibrium, are associated with hyperandrogenism. In our study mutant genotype (CC + GC) of IL-6 -G174C gene polymorphism were found significantly associated with high hirsutism score, higher total testosterone levels and oligomenorrhea in PCOS women. Moreover, hyperandrogenemic features were more commonly observed in women with metabolic syndrme as compared to without metabolic syndrome women in PCOS which could be because of Hyperinsulinemia [42] and hyperlipidemia is an excess of fatty substances called lipids largely cholesterol and triglycerides, in the blood. Hyperlipidemia can be of two types one is hypercholesterolemia in which there is a high level of cholesterol and the another one is hypertriglyceridemia, in which there is a high level of triglycerides [43] In conclusion the mutant C allele as well mutant genotype (CC+GC) of 174 IL-6 promoter gene were more associated with PCOS and with metabolic syndrome regardless of presence or absence of PCOS. This mutant genotype was also associated with high circulating IL-6 level, metabolic risk markers and hyperandrogenemic clinical and biochemical parameters in PCOS women and women with metabolic syndrome irrespective of presence or absence of PCOS, hence conclusively women with and without PCOS having mutant CC + GC genotype and mutant C allele might have high risk for developing metabolic syndrome. So the interactions among metabolic risk markers, insulin resistance and circulating IL-6 with 174 G/C IL-6 promoter gene polymorphism are undeniable.

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