SINGLE NUCLEOTIDE POLYMORPHISMS (CYP11 ALPHA AND CYP17) AND SERUM SEX HORMONE BINDING GLOBULIN LEVELS IN NORMAL AND POLYCYSTIC OVARY SYNDROME

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ABSTRACT

Objective: To examine the potential association between *CYP11 alpha* and *CYP17* polymorphism, and serum sex hormone binding globulin (SHBG) levels and their possible contribution to polycystic ovary syndrome.
Study Design: Case control study.
Place and Duration of Study: University of Health Sciences, Lahore Pakistan, from Jan to Oct 2017.
Methodology: A total of 60 cases of polycystic ovary syndrome and 60 controls and single nucleotide polymorphisms were studied for *CYP11 alpha (TTTTA)n* repeats and *CYP17* (-34 T/C) along with sex hormone binding globulin levels and testosterone levels. Data were collected through a specially designed questionnaire. Blood samples were collected followed by serum separation and deoxyribonucleic acid extraction using standard protocols. Serum was used to measure the levels of sex hormone binding globulin and androgens. Extracted deoxyribonucleic acid was screened for the polymorphisms by PCR. SPSS version 20 was used to calculate data statistics.
Results: Out of 120 total women, 60 were suffering from polycystic ovary syndrome. Amongst these 60 cases, 43 (71.7%) had changes in menstrual cycle, 36 (60%) with longer than 35 days (p=0.04). Acne (p<0.001), infertility (p=0.005), family history of polycystic ovary syndrome (p<0.001), family history of menstrual problem (p=0.005), changes in cycle (p=0.01), weight gain (p=0.03), anemia (p<0.001) and dyslipidemia (p<0.001) were significantly associated with polycystic ovary syndrome as compared to controls. Polycystic ovary syndrome was strongly associated with *CYP11 alpha* polymorphism (p<0.001) with an odds ratio (OR) of 45 (95% CI, 15-132).
Conclusion: *CYP11 alpha (TTTTA)n* repeats and *CYP17 (TT, TC, CC)* were significantly associated with polycystic ovary syndrome however both polymorphisms did not show any relationship to mean serum sex hormone binding globulin and testosterone levels.

Keywords: CYP11 alpha, CYP17, Polycystic ovary syndrome, Polymorphism, Sex hormone binding globulin, Testosterone.

INTRODUCTION

PCOS is of public health and clinical importance as it is quite common and affects one in five women of reproductive age[1]. It has been reported that the prevalence rate of PCOS in Pakistan is 20.7%[2]. It has quite prominent and diverse clinical impacts including reproductive (hyperandrogenism, infertility, hirsutism), psychological issues (increased anxiety, depressionand worsened quality of life) and metabolic (impaired glucose tolerance, insulin resistance, type 2 diabetes mellitus, deteriorating cardiovascular risk profiles)[3].

Among womenwithin reproductive-age the prevalence of PCOS is calculated at 4-12%[4]. A lot of genes from such pathways have been put to test including genes involved in steroid hormones synthesis and metabolism *(HSD17B1-3, StAR, CYP19, CYP11, CYP17)*. In previous few years, there are various developments in the understanding of pathophysiology and genetics of PCOS. *CYP11 Alpha* gene is a member of the cytochrome P450 family. It encodes many enzymes that are used in a variety of vital reactions in cell metabolism[5]. PCOS is found to be linked with the absence of four-repeat-units allele in polymorphic region of pentanucleotide *(TTTTA)n* repeats within *CYP11 Alpha* gene[6]. Cytochrome P450 17α-hydroxylase/17, 20-lyase *(CYP 17)* is an enzyme catalyzing two prominent activities, 17, 20-lyaseand 17α-hydroxylase is mandatory for the biosynthesis of gonadal and adrenalsteroids[7]. A polymorphism was found in the regulatory region of the *CYP17* gene, a T to C substitution-34 base pairs from the translation initiation point in the promoter region[8]. It has been said that this polymorphism may up-regulate the expression of *CYP17*, causing in an enhanced synthesis of androgens[9].

Sex hormone binding globulin (SHBG) is a protein which binds to both estradiol and testosterone. Its amount can vary in women with PCOS. If levels of the SHBG are either low or high, the amount of active testosterone can changeaccordingly[10]. In developed

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countries some work on these genes has been done and most of the researches deciphered these genes and make them candidate genes for PCOS. Especially, mutations in *CYP17* are supposed to contribute 75% susceptibility to the disease[11]. In this part of the world, the prevalence of this metabolic syndrome is high and more data are needed for planning the screening and early diagnosis of PCOS in women who are at higher risk. The present work will be useful in understanding the genotypic makeup of the Pakistani individuals and its relation with PCOS. Improving the classification of PCOS genotypes will undoubtedly improve calculating risk in siblings and thus in turn improving risk assessment accuracy and may facilitate genetic counseling.

METHODOLGY

It was a case control study conducted in the department of Physiology and Cell Biology, University of Health Sciences Lahore, from January to October 2017. It was conducted as per Helsinki declaration after approval by the Ethics Review Committee of University of Health Sciences Lahore. Convenient sampling technique was used.

Cases were females with PCOS between 20-40 years and healthy women matched for age were taken as normal.

Diagnosed patients of polycystic ovary disease were selected from outpatients departments of infertility clinics, Gynecology/Obstetrics wards of private and government tertiary care hospitals of Lahore and THQ of Chichawatni. Controls were taken from normal population and informed consent was taken from all the subjects. A questionnaire was given to all subjects to record their demographic features. All the subjects were assessed by taking complete medical history and physical examination. Following parameters were studied:

1. BMI
2. Serum SHBG levels
3. Serum Androgens
4. CYP11 alpha (TTTTA)n repeats
5. CYP17 (T to C substitution (34bp))

3ml venous Blood was collected from each subject and control in EDTA coated vacutainer tube and 2ml in serum vacutainer tube. DNA was isolated from whole blood of all the subjects by using standard phenol/chloroform method[23]. The extracted DNA was run on the 1.5% agarose gel (containing ethidium bromide) and seen under a UV trans illuminator to check its purity and integrity. The DNA samples were amplified by PCR using specifically designed primers. ELISA was done to measure levels of serum sex hormone binding globulin (SHBG) and serum testosterone.

The collected data were entered and analyzed using SPSS version 20. Mean and standard deviation were given for quantitative variables, frequencies, percentages and odd's ratios were given for qualitative variables (genotype). Normality of the data were checked by Shapiro-Wilk test. Chi-square test was applied to compare the SHBG level and genotype frequencies between the study and the control groups of the patients. Student t-test was used to compare mean values of BMI, age and the hormones between the different groups of females. *p*-value of <0.05 was considered statistically significant.

RESULTS

As per research protocol out of 120 subjects, 60 (50%) had PCOS and 60 (50%) were normal. The mean age of the study subjects (n=120) was 28.60 ± 6.14 years and mean age of menarche was 12.7 ± 0.49. Out of total women (n=120), 84 (70%) were married while 36 (30%) were unmarried; 23 (19.2%) had regular cycle while 97 (80.8%) with irregular cycle; two women (1.7%) had endometriosis also. Mean number of children bore by married women in the study group were 2.06 ± 1.60 with mean number of abortions were 0.19 ± 0.48 (Table-I).

Table-I: Association between SHBG and CYP17 polymorphism by independent sample t-test.

		SHBG		*p*-value
		Cases	Control	
CYP17 Polymorphis	Present	25.1 ± 15.17 (n=40)	17.50 ± 2.30 (n=5)	0.01
	Absent	25.93 ± 21.60 (n=4)	24.18 ± 15.06 (n=23)	0.51
	Total	60	60	

PCOS showed significant (p<0.001) difference with *(TTTTA)n* repeats in *CYP11 Alpha* gene with and a higher risk with Odds ratio of 19 (7-50). No repeat homozygous polymorphism and no repeat heterozygous polymorphism also differed significantly amongst cases and controls with *p*-values of <0.001 and 0.001 respectively. Likewise, both polymorphism showed a protective effect on presence of PCOS with Odds ratio if 0.179 (0.06-0.048) and 0.5 (0.04-0.339) (Table-II & III).

PCOS was significantly associated with allelic variant TT of *CYP17* gene (p<0.001) as compared to those subjects without PCOS having an OR of 10 (4-23) showing a very high risk of developing PCOS if this variation is present. Similar was the case of CC allelic

variant which was significantly (p=0.001) different between PCOS group and controls. TC allelic variation showed non-significant different with regards to its distribution amongst cases and control and OR was 1.4 (0.5-3.9) which again does not signify any increased risk (Table-IV & V).

Table-II: Association between PCOS and CYP11 Alpha polymorphism calculated according to the chi-square test with one degree of freedom (df=1).

		PCOS		*p*-value	ODD's Ratio 95% (CI)	Total
		Cases	Control			
CYP 11 Alpha Polymorphism	Present	n=60	n=10	<0.001	45 (15-132)	64
	Absent	n=0	n=50			56
	Total	60	60			120

Odds ratio (OR)= 45 (95%CI=15-132): p<0.001 (X^2=56.70, df=1)

Table-III: Association between PCOS and CYP17 polymorphism calculated according to the chi-square test with one degree of freedom (df=1).

		PCOS		*p*-value	ODD's Ratio 95% (CI)	Total
		Cases	Control			
CYP17 Polymorphism	Present	n=49	n=8	<0.001	28.9 (10.70-77.90)	57
	Absent	n=11	n=52			63
	Total	60	60			120

Odds Ratio OR=28.9 (95% C.I 10.70-77.90): p<0.001 (X^2 =56.7, df=1)

Table-IV: Distribution of subjects with (TTTTA)n repeats in CYP 11A1 amongst cases and control of PCOS.

Genotype	Cases n (%)	Controls n (%)	X^2	*p*-value	ODD's Ratio (95% CI)
(TTTTA) n Repeat	49 (81.6)	11 (18.3)	48.10	<0.001	19 (7-50)
No repeat homozygous	6 (10)	23 (38.3)	13.14	<0.001	0.179 (0.06-0.048)
No repeat Heterozygous	5 (8.3)	26 (43.3)	19.10	<0.001	0.5 (0.04-0.339)

Table-V: Distribution of genotypic variation in subjects with CYP17polymorphism amongst cases and control of PCOS.

Genotype	Cases n (%)	Controls n (%)	X^2	*p*-value	ODD's Ratio (95% CI)
TT	43 (71)	12 (20)	32.25	<0.001	10 (4-23)
TC	11 (18.3)	8 (13.3)	0.56	0.61	1.4 (0.5-3.9)
CC	6 (10)	40 (66)	40.20	<0.001	0.5 (0.02-0.15)

DISCUSSION

PCOS remains a heterogeneous condition with multipronged affects that extends from reproductive problems to metabolic derangements; from physical mal-attributes to psychological repercussions[12]. Ongoing research continues to dig into both preventable etiological causes to best treatment modalities with favorable disease outcomes and negligible adverse effects profile and ultimately cause a positive change in the quality of life of the sufferers. As the prevalence of disease is on the rise, a vast majority of women across different cultures, races and parts of the world continue to suffer from this condition whose implications are so comprehensive in affecting one's life[13]. Study of genetic attributes of PCOS has opened up a new promising arena for researchers who want to study etiological factors of this syndrome[14]. In the present study, polymorphisms in *CYP11 Alpha* and *CYP17* genes which are involved in steoidogenesis and the serum levels of SHBG and testosterone were studied alongside other parameters of PCOS.

This study showed that out of the women suffering from PCOS (cases) (n=60), 43 (71.7%) (p=0.01) had changes in menstrual cycle, 36 (60%) had longer than 35 days (p=0.04) and 9 (22.5%) were infertile (p=0.005) showing a significant association with the classical hallmarks of the disease as compared to control group. These features are consistent with various studies conducted on patients with PCOS[14]. The same study also observed that infertility was very common in patients with PCOS, 90-95% of women presenting with anovulation are having PCOS.

The PCOS patients in this study showed high frequency of metabolic derangements i.e. 53 (88.3%) of 60 women experienced weight gain. Out of these women, weight gain (p=0.03) was significantly associated with PCOS. Different studies have shown strong prevalence of dyslipidemia and obesity with PCOS and their deleterious effects on patients[15]. The overwhelming cases 54 of 60 (9%) PCOS, in this study had hirsutism and 26 (43.3%) had acne (p<0.001). A previous studystated higher frequency of hirsutism in women with PCOS[16]. Family history of PCOS (p<0.001) and menstrual problems (p=0.005) showed highly significant association with PCOS endorsing the role of genetic and familial factors as shown by a previous study[17].

It was found in the this study that PCOS was strongly associated with *CYP11 Alpha (TTTTA)n* polymorphism with an Odds Ratio (OR) of 45 (95% CI=15-132) with highly significant (p<0.001) difference between cases and controls (calculated value of chi square (X^2) as 64.8). PCOS showed significant (p<0.001) difference with *(TTTTA)n* repeats in *CYP11 Alpha* gene with higher risk (Odds ratio=19; CI=7-50) of PCOS. Homo-

zygous (no repeats) and no repeat heterozygous status also differed significantly among cases (p<0.001) and controls (p=0.001) respectively. Likewise, both showed a protective effect on presence of PCOS with weak Odds ratio=0.179 (0.06-0.048) and 0.5 (0.04-0.339). Aprevious large-scale analysis of *CYP11 Alpha* showed non-significant difference in *(TTTTA)n* repeat in cases and controls[18]. However, another study observed a significant difference in *(TTTTA)n* repeats among cases and controls which is in agreement with our results[19]. We recommend that further studies be carried out on local women to settle this point.

Various studies have reported raised *CYP17* expression and the enzymatic activity in theca cells of ovary from patients with PCOS and also increased transactivation of the *CYP17* promoter[20]. Research work has shown that CYP17 expression is deregulated at the level of mRNA stability in theca cells with PCOS[21]. Similarly, in our study, CYP17 polymorphism was highly significant (p<0.001) in cases when compared with controls with Odds Ratio (OR=28.9; 95% CI=10.7-77.9) and chi square (X^2) value of 56.7.

On the other hand our study showed very strong relationship between having polymorphism in *CYP17* and women having PCOS. Earlier it was observed that there is increased *CYP17* polymorphism in women having PCOS but this study lacked comparison with controls and cannot be used for concrete evidence[22]. Moreover, they did not undertake hormonal assays of testosterone in these patients and failed to comment on relationship between this polymorphism and hyperandrogenism. Earlier it was reported that this enzyme's up regulation may lead to increased androgen synthesis[23]. It can be concluded from above discussion that *CYP17* polymorphism may have association with PCOS but it does not leads to dysregulation of 17, 20 enzyme activity and cannot be linked to increased androgen production in patients of PCOS.

Sex hormone binding globulin (SHBG) is a protein that binds to both testosterone and estradiol. Our results showed a negative correlation between sex hormone binding globulin and serum testosterone level meaning that higher the level of sex hormone binding globulin (SHBG) the lower was the level of testosterone. A previous study also stated that SHBG is associated inversely with free testosterone[23].

This study thus concludes that *CYP17* polymorphism is strongly associated with SHBG levels (p= 0.01). Such observation was contradictory to the result documented by a previous study which did not find any association between SHBG levels and *CYP17* polymorphism, though this research was done among breast cancer survivors[24]. PCOS was significantly associated with TT genotype of CYP17 gene (p=0.0001) as compared to those subjects without PCOS and having an OR of 10 (CI=4-23) showing a very high risk of developing PCOS if this variation is present. Similar was the case with CC genotype with p=0.001, which was significantly different in PCOS group and controls however the OR was O.5 (CI=0.02-0.15) therefore showing no increased risk. In fact presence of this allele points towards a decreased risk. Another study also did not find any significant difference in cases and controls with a lower frequency of CC as compared to TC as seen in our findings. It was previously observed that there is a significant difference in TC polymorphism which is contrary to our findings, however, the difference was small and was 8% in cases as compared to 0% in controls[24].

The results of the present study clearly showed that to help the patients of PCOS, there is a need to repeat this study of Pakistani women by increasing the number of samples, other candidate genes and hormonal levels. It is also submitted that we are a genetically distinct group from Caucasian population on which major studies have been done, and this type of study should also include local population from Baluchistan, KPK and Sindh as this study was performed with Punjabi population.

CONCLUSION

There is a strong association of polymorphism of *CYP11 Alpha* gene with PCOS but the hyperandrogenemia seen in patients with PCOS might not be a result of this genetic polymorphism. However, mean serum testosterone and sex hormone binding globulin levels in women with and without this polymorphism show no significant difference. And there is no association between the above-mentioned polymorphism and hypernadrogaemia seen in PCOS.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any authors.

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