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Original article

Association of Angiotensin-Converting Enzyme gene polymorphism in Pakistani women with the atypical steroidogenesis in Polycystic ovarian syndrome: A case-control study

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ABSTRACT

Background: Polymorphism in the angiotensin-converting enzyme gene (ACE) is responsible for elevated ACE concentrations in plasma. High ACE levels induce insulin resistance and hyperandrogenism, which are the main attributes of polycystic ovary syndrome (PCOS). Therefore, it was hypothesized that I/D polymorphism plays a role in the pathogenesis of PCOS.
Objective: A case-control study was designed to investigate the association of I/D polymorphism of the ACE gene with PCOS in Pakistani women of reproductive age.
Methods: ACE I/D polymorphism was assessed in 252 women of age group 16–40 years. For genotypic analysis, PCR amplification of genomic DNA was carried out. Statistical analysis was performed to interpret the results using SPSS software.
Results: Our study showed that PCOS women were more likely to have a high body mass index and waist circumferences. Most PCOS patients had menstrual irregularities 99.3%, hirsutism 75.2% and cysts in ovaries 66.6%, along with other hyperandrogenic conditions (P-value = 0.001). The genotypic and allelic frequencies were significantly different between patients and controls. There was a significant association of three genotypes with the ratio of LH: FSH among PCOS patients (P = 0.05). Anthropometric characters, comorbidities, clinical symptoms, and PCOS conditions showed no statistical significance with ACE polymorphism.
Conclusions: ACE I/D polymorphism was not found associated with clinical conditions of PCOS in women of reproductive age. However, it was associated with atypical steroidogenesis. So, it indicates that ACE I/D polymorphism aggravates the pathogenesis of PCOS.

1. Introduction

Polycystic Ovarian Syndrome (PCOS) has become a common problem among women of reproductive age in Pakistan and affects 8–12% of the world's population (Donà et al., 2017). It is a complicated endocrinopathy with several adverse effects on women health (Abinaya et al., 2019). Prominent diagnostic features are polycystic ovaries, hyperandrogenism, high luteinizing hormone (LH) levels, insulin resistance (IR), menstrual disorder and hirsutism (Lone et al., 2020; Singh et al., 2019). Both environmental and genetic risk factors tremendously contribute to the development of broad-spectrum metabolic defects of PCOS (Merkin et al., 2016). In previous research, genes involved in ovarian and adrenal steroidogenesis, insulin action, energy homeostasis, and chronic

Abbreviations: ACE, Angiotensin-converting enzyme; PCO, Polycystic ovarian syndrome; LH, Leutinizing hormone; FSH, Follicular stimulating hormone; IR: Insulin resistanceRAS, Renin-Angiotensin System; A-II, Angiotensin-II; ROS, Reactive oxygen specie; BMI, Body mass index; WC, Waist circumference; CVD, Cardiovascular diseases; Vit, Vitamin; Ca, Calcium; EDTA, Ethylenediamine tetraacetic acid; SPSS, Statistical Package for Social Sciences; HWE, Hardy-weinberg equilibrium; PCR, Polymerase chain reaction.

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inflammation were mainly focused on PCOS candidate genes (Dadachanji et al., 2018; Lone et al., 2020a).

Renin-Angiotensin System (RAS) plays a crucial role in body homeostasis, oocyte maturation, ovulation, the formation of corpus luteum, and steroidogenesis. It is expressed in various tissues, including the ovary (Bayram et al., 2011). ACE, also called peptidyl dipeptidase A or kininase II, acts as a main rate-limiting component of the RAS system. The ACE gene encodes it on chromosome 17. The ACE product, Angiotensin II (Ang II) contributes to the many physiologic processes such as steroidogenesis (Bayram et al., 2011; Bernstein et al., 2013). Besides, its elevated concentrations are suspected to be involved in the pathogenesis of obesity, inflammation, oxidative stress, insulin resistance and PCOS (Ramalingam et al., 2017). However, a few studies have observed the role of ACE in hormonal imbalance. The presence of the ACE gene with insertion-deletion polymorphism (I/D polymorphism), based on the insertion of 287 bp sequence, is associated with elevated ACE levels in plasma. Subsequently, it upregulates the RAS and leads to PCOS development by disrupting the normal steroidogenesis (Palumbo et al., 2016). Thus, it is of interest for researchers to evaluate the role of ACE in the pathophysiology of PCOS.

Many studies revealed that high concentrations of ACE responsible for insulin resistance (IR) by producing reactive oxygen species (ROS) in tissues (Manucha et al., 2015; Ramalingam et al., 2017). IR provokes hyperandrogenism that is a significant manifestation of PCOS phenotypes. I/D polymorphism in the ACE gene induces low-grade inflammation due to ROS. IR and hyperandrogenism are the metabolic risk factors of the deadly quartet (obesity, diabetes mellitus, etc.) and associated with PCOS pathophysiology (Barrea et al., 2018). Previously we studied the associations of pathological causes of PCOS with different genes (Lone et al., 2020a,b). Here, we aimed to investigate the association of I/D polymorphism of the ACE gene with the risk of polycystic ovary syndrome by disrupting the steroidogenesis process.

2. Methods

2.1. Study design and setting

A total of 252 subjects were recruited from the gynaecology outpatient clinic, Jinnah Hospital, Lady Willingdon Hospital, CRC labs and Institute of Microbiology & Molecular Genetics, University of the Punjab Lahore. Ethical approval for the study was taken from CRC Labs (Ethical Review Board Ref # 26-17/ERB/CLRC/ 27th; dated 28-07-2016). The study subjects included were 162 PCOS patients and 90 healthy women. All participants provided written, informed consent with information about the research project. Written informed consent was taken from the parents or guardians of the participants under 18 years of age.

2.2. Inclusion and exclusion criteria

Rotterdam diagnostic criteria 2003 (ESHRE & Group, 2004) was used to select the PCOS cases. Healthy subjects with the normal menstrual cycle, ovaries without any cyst and having no signs of hyperandrogenism were included as controls. The age of participants of the study ranged between 16 and 40 years. Women on hormonal medication and infectious diseases, hypothyroidism, androgen-secreting cancers, adrenal hyperplasia, insulin resistance or hyperglycemia, advanced stage (III or IV) endometriosis or suffering from premature ovarian failure were excluded from the study.

2.3. Clinical evaluation

All participants filled a survey-based questionnaire. It included the information of sociodemographic characters, age, height, weight, body mass index (BMI), waist circumference (WC), fasting glucose level (above 100 mg/dL), menstrual cycle and its irregularity, hirsutism, acne, weight gain, skin discolouration, mood swings, patient's history of metabolic disorders as diabetes, hypertension, cardiovascular diseases (CVDs), vitamin and calcium deficiency (Vit/Ca deficient), use of fertility medicines and oral contraceptives and family history of PCOS, diabetes, hypertension, cardiovascular diseases and Vit/Ca deficiency. ELISA kits (Accesss 2 beckman coulter) were used to measure Leutinizing hormones (LH) and follicle-stimulating hormones (FSH). The ratio of 1:1 for LH: FSH was considered normal.

2.4. Genotype determination

A volume of 5 ml of peripheral blood was collected on the 2nd or 3rd day (PCOS guidelines, 2012) of the menstrual cycle. It is equally aliquoted into EDTA and serum vials and stored at −20℃ for genomic studies and serum analysis. In nested PCR, triple primers were used to observe the ACE polymorphism. A reaction mixture of 20 µl contained forward primer **5′**TCCCATCCTTTCTC CCATTTCT'3, 0.6 µl, reverse primer CATGCCCATAACAGG TCTTCAT'3 0.6 µl and internal nested primer **5′** GGTTTCACCGTTTTAGCCG'3 (Nawaz and Hasnain, 2009) 0.6 µl, master mix 10 µl, nuclease-free water 6.2 µl and DNA template 2 µl. PCR program was developed for 35 cycles with denaturation at 94 °C for 30sec, annealing at 58 °C for the 30sec, and extension at 72 °C for 30sec in a Bio-Rad T100 thermal cycler. Bands of amplified products were visualized on 2% agarose gel under UV illuminator. The PCR results were evaluated by observing the bands as 210 bp fragment for DD, 498 and 264 bp fragments for II, and 498, 264, 210 bp fragments for ID genotype.

2.5. Statistical analysis

Statistical Package for Social Sciences (SPSS, IBM statistics) version 22, MEDCALC online were used for statistical analysis. Data were evaluated by calculating mean, standard deviation, percentages, odd ratio, independent T-test and Chi-square values. Alleles and genotypes were following Hardy-Weinberg equilibrium (HWE), calculated using an online calculator (http://apps.biocompute.org.uk/hwe-mr-calc.html) . The level of significance was <0.05. For correlation analysis, the Pearson correlation was applied.

3. Results

3.1. Baseline characteristics

The anthropometric variables, comorbidities, clinical characteristics in PCOS and non-PCOS groups are shown in Table 1. PCOS women were older than controls with mean ages of 25.21 ± 4.57 0 years and non-PCOS 22.9663 ± 4.239 years. Means of the weight of PCOS patients were tremendously high 68.45 ± 14.18 as compared to controls 53.84 ± 8.54. There was a difference between the mean values of BMI of patients 27.694 ± 5.770 and controls 20.17 ± 6.315. In Fig. 1, the box plot's median values represent the inclination of age and BMI among the two groups. WC of PCOS was also slightly higher than the healthy women. These differences in the mean ± standard deviation values were significant statistically between experimental and control groups regarding age, weight, BMI and WC except height and fasting glucose level. However, diabetic patients had a high fasting glucose level.

Table 1
Anthropometric and clinical data of PCOS and non-PCOS group.

Variables	PCOS (n = 162)	Controls (n = 92)	P-value
Anthropometric Characters			
Age	25.21 ± 4.570	22.9663 ± 4.23849	0.0001*
Height	1.6687 ± 0.544	1.59 ± 0.064	0.1685
Weight	68.45 ± 14.18	53.84 ± 8.54	0.0001*
BMI	27.694 ± 5.770	20.17 ± 6.315	0.0001*
WC	0.82 ± 0.084	0.8 ± 0.021	0.0258*
Fasting Glucose level	100 ± 24.07	96.70 ± 11.59	0.2019
Diagnostic criteria characteristics			
Ultrasound scan Confirmed	108(66.6%)	6(0%)	0.0001*
Irregular periods	161 (99.3%)	3(3.3%)	0.0001*
Facial hairs	121(75.2%)	4(4.3%)	0.00*
Biochemicals tests			
Elevated LH: FSH	102 (63%)	33(35.9%)	0.0001*
Comorbidities			
Hypertensive	64(39.5%)	4(4.3%)	0.0001*
Diabetes	7(4.32%)	2(2.17%)	0.3738
CVDs	1(0.61%)	0(0%)	0.4538
Vit/Ca deficient	12(7.4%)	0(0%)	0.0076*
Family history of other diseases			
Hypertension	88(54.32%)	11(11.9%)	0.0001*
Diabetes	86(53%)	25(27.1%)	0.0001*
CVDs	16(9.8%)	3(3.2%)	0.0541
Vit/Ca deficiency	1(0.61%)	0(0%)	0.4538
PCOS	41(25.3%)	0(0%)	0.0001*
Other clinical conditions			
Rapid Gaining weight	136(85.5%)	5(5.4%)	< 0.0001*
Skin coloration	76(47.5%)	4(4.3%)	< 0.0001*
Acne	104(64.6%)	12(13%)	< 0.0001*
hungry/fatigue	132(81.4%)	0 (0%)	< 0.0001*
Mood swings	130(80.2%)	16(17.4%)	< 0.0001*
Infertility	91(56.13%)	5(5.4%)	< 0.0001*
Surgery history	26(16.1%)	3(3.2%)	0.0019*
Taking contraceptives	26(16.0%)	0(0%)	< 0.0001*

* indicate the level of significance $P \leq 0.05$.

Among the clinical features of criteria for assessing the PCOS, ovaries with multiple cysts, irregular menstrual cycle and hirsutism were significantly high in PCOS vs non PCOS women (P-value < 0.05) (Table 1). Frequencies of comorbidities as hypertension, diabetes, CVDs and Vit/Ca deficiency among PCOS women were higher than controls. More than half of PCOS women had family histories of hypertension and diabetes. About one-quarter of the study population of patients told the presence of the PCOS in their families. These differences in the frequencies of hypertension and Vit/Ca deficiencies and family histories of hypertension, diabetes and Vit/Ca deficiencies were significant statistically among the two groups.

As for other PCOS outcomes, rapid weight gain, skin discolouration, acne, fatigue, mood swings, infertility, and surgery history were common in PCOS patients with P-value < 0.05. At the same time, the use of oral contraceptives was not a significant risk factor of PCOS (P-value > 0.05) (Table 1).

3.2. ACE I/D polymorphism and PCOS

Three different genotypes were observed on agarose gel; 210 bp fragment for DD; 498 and 264 bp fragments for II; and 498, 264, 210 bp fragments for ID genotype as shown in Fig. 2.

The allelic and genotypic frequencies in patients and controls, along with the ACE polymorphism percentages, is shown in Table 2, by using different penetrance models. The P-value (0.005) for the additive model showed that polymorphism at ACE was significantly associated with atypical steroidogenesis in PCOS women. The additive model shows a high allelic frequency for allele I in controls, whereas allele D has the highest frequency in patients. The codominant model reports the highest genotypic frequency for heterozygous genotype (ID) in controls vs patients; II and DD genotypic frequency is higher in patients. Furthermore, the genotypic frequencies for patients and controls are nearly similar for patients and controls in dominant and recessive models.

A significant association was found between three genotypes of ACE and Leutinizing hormone: Follicle-stimulating hormone (LH: FSH) ratio in the PCOS subjects ($P \leq 0.05$), using ANOVA analysis. It was observed that both patients and controls with DD genotypes had a higher LH: FSH ratio, contrary to those having and ID and II genotypes. In controls, the distribution of genotypes was not associated with LH: FSH ratio (P = 0.5) (Table 3). Also, the genotypes did not show a significant association with anthropometric characteristics, comorbidities, clinical conditions and other related parameters of PCOS (P-value > 0.05). Pearson correlation between LH: FSH ratio and PCOS patients' clinical parameters were determined in both patients and the control group. A positive correlation was revealed between patients with diabetes and their LH: FSH ratio (r = 0.199*, P-value 0.047). In controls, infertility showed a negative correlation with LH: FSH ratio (r = -0.0321, P-value 0.023). No significant correlation was found in ACE genotypic distribution regarding age, BMI, waistline, hypertension, family history of hypertension, diabetes and PCOS, irregular menstrual cycle, hirsutism, gaining weight, acne, and found (Table 4).

4. Discussion

This study showed the means of body weight, BMI and WC were significantly higher in PCOS compared to healthy women. This exhibits the role of obesity in the development of clinical features of PCOS. The research revealed that the excess of adipose tissue downregulates the adipokines necessary for insulin sensitivity in

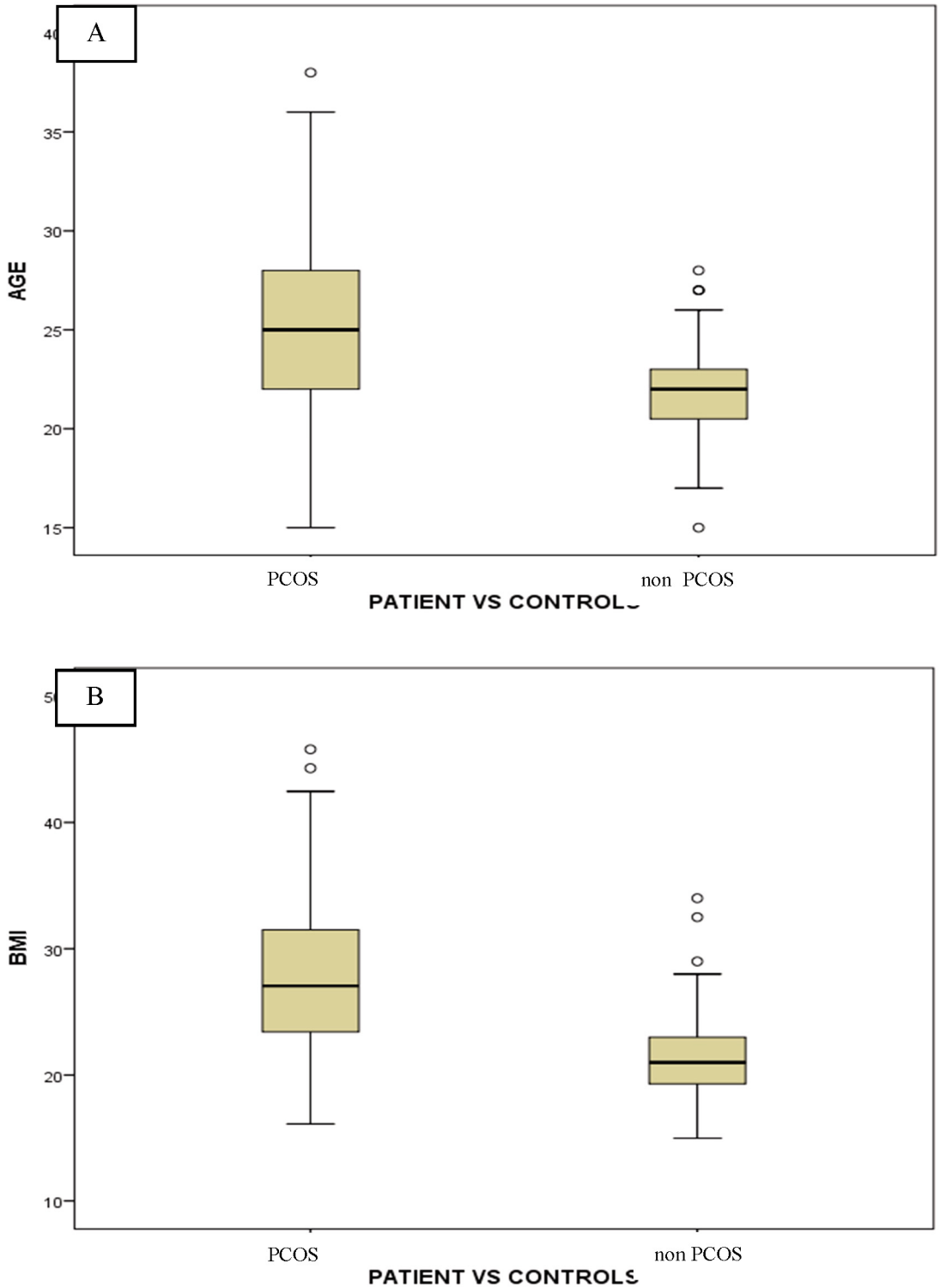

Fig. 1. The box plot (A) indicates maximum, minimum, median values of age, along with lower and higher quartile among PCOS and non PCOS women. (B) This represents the maximum, minimum, median, and lower and higher quartile values for BMI among PCOS and non PCOS women.

PCOS. A case-control study from Iran revealed high BMI and WC in PCOS diagnosed women (Behboudi-Gandevani et al., 2017). Another research from India was in accordance with our study, also obtained 1.74 times high BMI in PCOS than non-PCOS (Begum et al., 2017).

In our results, PCOS patients with hypertension, vitamin and calcium deficiencies were significantly high in number. Also, the family histories of hypertension, diabetes and PCOS were common among patients. In previous studies, hypertension was presented as a PCOS risk factor and highly prevalent in women with PCOS (Ollila et al., 2018). In Moldova, about half of the PCOS patients had a family history of mothers suffering from PCOS (Voloceai, 2017). Deficiencies of vitamins and calcium may be triggering hormonal imbalances in the body. The beneficial effects of the co-supplementation of vitamins and calcium on hormonal profile in PCOS women show their role in the pathogenesis of the disorder.

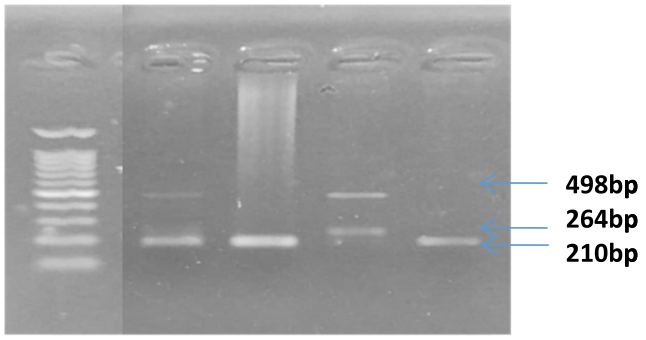

Fig. 2. Bands of ACE polymorphic alleles, lane 1 contain ladder of 100 bp, lane 2 with 3 bands of 498 bp, 264 bp 210 bp indicate the ID genotype, lane 3 and 5 with one band of 210 bp indicate the DD genotype, Lane 4 with 2 bands of 498 bp, 264 bp indicate II genotype.

According to prior studies, women with PCOS were more likely to have obesity, hypertension, diabetes mellitus (T2DM) and cardiovascular disorders (Fauser, 2018; Fernandez et al., 2018).

Hyperandrogenic conditions like acne, skin discolouration, extreme hunger, fatigue, mood swings and psychological issues were common among PCOS women. A large amount of relevant literature revealed that women with PCOS were more prone to have these clinical conditions due to elevated androgen (Dashti et al., 2019; Lopes et al., 2018; Stapinska-Syniec et al., 2018). As an indication of the association of PCOS with the absence of pregnancy, more than half of the cases were facing infertility. Balen and Morley reported that approximately 80% of women who were suffering from anovulatory infertility had PCOS (Balen et al., 2016; Fauser, 2018; Fernandez et al., 2018).

The genotypic analysis revealed the high prevalence of allele D and genotype DD in the PCOS group. In controls, allele I and II genotype were observed more often than DD. High percentages of DD genotype in patients were reported in PCOS patients with hyperandrogenism (Cintra et al., 2018; Deepika et al., 2013). By further analysis, we found the differences in genotypic distributions and allelic frequencies were significant. Although no significant association of ACE I/D polymorphism with PCOS phenotypic characteristics was found, yet the LH: FSH ratio in the PCOS varied among the three genotypes in cases significantly ($P \leq 0.05$). This ratio was found significantly higher in DD genotype than other genotypes in both patients and control. It indicates that elevated ACE levels in the plasma due to I/D polymorphism may be responsible for hyperandrogenism in PCOS patients. Ang II, the ACE product, inhibits the progesterone secretions that give rise to the secretion of LH through a negative feedback mechanism (Che et al., 2009b). An increase in LH and reduced or normal secretions of FSH stimulate androgen production in the ovary (Kumar et al., 2016). It is evident from an observational study in which androgen levels decrease after treatment with the angiotensin-converting enzyme inhibitor for four weeks (Connolly et al., 2018). This implicates the fact that polymorphism may cause a surge of LH, leading to PCO (Che et al., 2009a; Hashemi et al., 2017). These findings suggest that I/D polymorphism leads to abnormal RAS activity and is associated with the onset of metabolic disturbances, glycemic dysregulation, PCOS and other clinical consequences of PCOS such as hypertension and insulin resistance (Cintra et al., 2018). Another interesting finding of our research was the positive correlation of diabetes in PCOS women with their abnormal LH: FSH ratio. The high prevalence of Diabetic Mellitus type 2 in PCOS women may be due to the direct relation of insulin resistance with the LH: FSH ratio (Smaism et al., 2016). A follow-up study over 1919 person-years observed PCOS as a significant risk factor of Diabetic Mellitus type 2 in women of reproductive age (Kakoly et al., 2018).

Pakistan has scarce data published on PCOS and its phenotypic and genotypic pathologies, while no work yet has been reported

Table 2
Different penetrance models showing genotypic and allelotypic frequencies for ACE gene polymorphism.

SNP	Patients %	Control%	OR (95% CI)	p-value
Codominant				
II	58 (36.02)	40 (0.44)	1.62 (0.96, 2.71)	0.064
DD	91 (0.57)	35 (0.38)		
ID	12 (0.07)	16 (0.16)		
Additive				
I	122 (0.39)	88 (0.53)	0.57 (0.39, 0.84)	0.0050*
D	188 (0.61)	78 (0.47)		
Dominant				
DD	90 (0.56)	40 (0.44)	1.62 (0.96, 2.71)	0.0878
II + ID	71 (0.44)	51 (0.56)		
Recessive				
II	58 (0.56)	35 (0.38)	0.90 (0.53, 1.53)	0.7859
DD + ID	103 (0.64)	56 (0.62)		

Frequencies are represented as number and percentage; p-Values were calculated using Chi-square test; p-values < 0.05 were considered statistically significant; n, Number of subjects; OR, Odds ratio; CI, Confidence interval.

Table 3
Association of ACE polymorphism with LH: FSH ratio.

		PCOS			Non-PCOS		
	Genotype	N	Mean ± S. D	P- value	N	Mean ± S. D	P-value
LH: FSH	II	39	0.28 ± 0.46	0.050*	32	0.76 ± 0.83	0.5
	DD	59	0.53 ± 0.50		20	1.143 ± 0.95	
	ID	4	0.50 ± 0.58		14	0.80 ± 0.92	
	Total	102	0.43 ± 0.5		66	0.88 ± 0.88	

* indicate the level of significance $P \leq 0.05$.

Table 4
Pearson correlation between LH: FSH ratio and other parameters of PCOS.

Series no.	Parameters	PCOS		Non-PCOS	
		Correlation 'r'	P-value	Correlation 'r'	P-value
	LH: FSH ratio	1.00	0.00	1.00	0.00
1	Age	−0.186	0.066	0.059	0.687
2	BMI	−0.035	0.738	0.099	0.564
3	Waist line	−0.074	0.476	0.057	0.693
4	Hypertension	−0.067	0.508	0.022	0.884
5	Diabetes	0.199*	0.047	0.007	0.960
6	Family history of Hypertension	−0.097	0.335	0.016	0.912
7	Family history of Diabetes	0.136	0.178	−0.040	0.784
8	Family history of PCOS	0.002	0.986	−0.045	0.776
9	Irregular periods	−0.035	0.733	−0.054	0.710
10	Hirsutism	0.168	0.098	0.011	0.942
11	Gaining weight	−0.025	0.810	−0.084	0.560
12	Acne	0.060	0.559	−0.277	0.052
13	Infertility	−0.059	0.576	-0.0321*	0.023
14	Genotyping ACE	−0.172	0.091	−0.104	0.482

Cannot be computed because atleast one of variables is constant.
Correlation is significant at the level of 0.05* (2-tailed).
Correlation is significant at the level of o.oq** (2-tailed).

regarding the association of ACE polymorphisms with PCOS. The significance of our research is presenting the association of ACE gene polymorphism with hormonal parameters of PCOS in Pakistani women. This represents the growing interest in the importance of gene polymorphisms in the pathogenesis of PCOS. However, a short period of research limits the sample size of the study. Also, the participation of first-degree relatives of the subjects could enhance the significance of this study. New strategies are needed to screen all subjects for genetic alterations of PCOS pathogenesis. This will help prevent the disease, transition to self-management, and improve women's health in Pakistan.

5. Conclusions

In conclusion, I/D polymorphism was significantly associated with atypical LH: FSH ratio in PCOS patients of the Pakistani population. Therefore, the presence of I/D polymorphism suspects to affect the steroidogenesis process, which in turn can trigger the development of PCOS in women of reproductive age. It is suggested that ACE I/D polymorphism may play a role in the pathogenesis of the disease, while it is not the main etiological factor of PCOS.

Ethical approval and consent to participate

Ethical Review Boad of Citi Lab and Research Centre approved this work under Ref # 26-17/ERB/CLRC/ 27th. All the required material for project was evaluated by committee including questionnaire and consent form.

Consent for publication

All the authors consented for the paper publication.

Availability of data and material

The analysed data for the current study will be available from the corresponding author.

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Authors contribution

KN designed the study, designed the questionnaire, collected data from participants, applied statistics and drafted this manuscript. SR contributed to study design and helped in finalizing the manuscript. NML participated in questionnaire designing, statistical analysis and finalizing the manuscript. SS and AEZ helped in sample collection from respective OPDS and reviewed the final draft. SKS participated in drafting the manuscript. All authors critically reviewed the manuscript and approved the final version.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Abinaya, S., Siva, D., Sabitha, R., Achiraman, S., 2019. An overview of hyperandrogenism in PCOS and the prospective underlying factors. Res. J. Life Sci. Bioinform. Pharmac. Chem. Sci. 1 (5), 179–186.
Balen, A.H., Morley, L.C., Misso, M., Franks, S., Legro, R.S., Wijeyaratne, C.N., Teede, H., 2016. The management of anovulatory infertility in women with polycystic ovary syndrome: an analysis of the evidence to support the development of global WHO guidance. Hum. Reprod. Update 22 (6), 687–708.
Barrea, L., Marzullo, P., Muscogiuri, G., Di Somma, C., Scacchi, M., Orio, F., Savastano, S., 2018. Source and amount of carbohydrate in the diet and inflammation in women with polycystic ovary syndrome. Nutr. Res. Rev. 31 (2), 291–301.
Bayram, B., Kılıççı, Ç., Önlü, H., Özkurt, M., Erkasap, N., Yıldırım, E., Şahin, F., 2011. Association of angiotensin converting enzyme (ACE) gene I/D polymorphism and polycystic ovary syndrome (PCOS). Gene 489 (2), 86–88.
Begum, G.S., Shariff, A., Ayman, G., Mohammad, B., Housam, R., Khaled, N., 2017. Assessment of risk factors for development of polycystic ovarian syndrome. Diabetes 4 (1), 164–167.
Behboudi-Gandevani, S., Tehrani, F.R., Yarandi, R.B., Noroozzadeh, M., Hedayati, M., Azizi, F., 2017. The association between polycystic ovary syndrome, obesity, and the serum concentration of adipokines. J. Endocrinol. Invest. 40 (8), 859–866.
Bernstein, K.E., Ong, F.S., Blackwell, W.-L.B., Shah, K.H., Giani, J.F., Gonzalez-Villalobos, R.A., Fuchs, S., 2013. A modern understanding of the traditional and

nontraditional biological functions of angiotensin-converting enzyme. Pharmacol. Rev. 65 (1), 1–46.
Che, Y., Cao, Y., Wu, X., Sun, H.-X., Liang, F., Yi, L., Wang, Y., 2009a. Association between ACE gene I/D polymorphisms and hyperandrogenism in women with polycystic ovary syndrome (PCOS) and controls. BMC Med. Genet. 10 (1), 1–6.
Che, Y., Cao, Y., Wu, X., Sun, H.-X., Liang, F., Yi, L., Wang, Y., 2009b. Association between ACEgene I/D polymorphisms and hyperandrogenism in women with Polycystic Ovary Syndrome (PCOS) and controls. BMC Med. Genet. 10 (1), 64.
Cintra, M.T.R., Balarin, M.A.S., Tanaka, S.C.S.V., Silva, V.I.M.D., Marqui, A.B.T.D., Resende, E.A.M.R.D., et al., 2018. Polycystic ovarian syndrome: rs1799752 polymorphism of ACE gene. Revista da Associação Médica Brasileira 64 (11), 1017–1022.
Connolly, A., Leblanc, S., Baillargeon, J.-P., 2018. Role of lipotoxicity and contribution of the renin-angiotensin system in the development of polycystic ovary syndrome. Int. J. Endocrinol. 2018.
Dadachanji, R., Shaikh, N., Mukherjee, S., 2018. Genetic variants associated with hyperandrogenemia in PCOS pathophysiology. Gen. Res. Int. 2018, 1–12.
Dashti, S., Abdul Hamid, H., Mohamad Saini, S., Shah Abu Bakar, A., Sabri, B., Inani, N. A., et al., 2019. Prevalence of polycystic ovary syndrome among Malaysian female university staff. J. Midwifery Reprod. Health 7 (1), 1560–1568.
Deepika, M., Reddy, K.R., Rani, V.U., Balakrishna, N., Latha, K.P., Jahan, P., 2013. Do ACE I/D gene polymorphism serve as a predictive marker for age at onset in PCOS?. J. Assist. Reprod. Genet. 30 (1), 125–130.
Donà, S., Bacchi, E., Moghetti, P., 2017. Is cardiorespiratory fitness impaired in PCOS women? A review of the literature. J. Endocrinol. Invest. 40 (5), 463–469.
ESHRE, T. R., & Group, A.-S. P. C. W., 2004. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil. Steril. 81 (1), 19–25.
Fauser, B.C., 2018. PCOS: Implications of Cardiometabolic Dysfunction *Pre-Menopause, Menopause and Beyond*. Springer, pp. 119–126.
Fernandez, R.C., Moore, V.M., Van Ryswyk, E.M., Varcoe, T.J., Rodgers, R.J., March, W. A., Davies, M.J., 2018. Sleep disturbances in women with polycystic ovary syndrome: prevalence, pathophysiology, impact and management strategies. Nature Sci. Sleep 10, 45.
Hashemi, A.H., Mozdarani, H., Mozdarani, S., 2017. The relationship between hormones level and body mass index with insertion and deletion (D/I) polymorphism of ACE gene in infertile patients with polycystic ovary syndrome. [Gene polymorphism, polycystic ovary syndrome (PCOS), Angiotensin converting enzyme gene (ACE gene), Infertility]. 2017, vol. 6, no. 1, pp. 7. https://doi.org/10.15562/bmj.v6i1.382.
Kakoly, N.S., Earnest, A., Teede, H.J., Moran, L.J., Joham, A., 2018. The Impact of Obesity on the Incidence of Type 2 Diabetes Mellitus Among Women with Polycystic Ovary Syndrome. Available at SSRN 3207917.
Kumar, A.N., Naidu, J.N., Satyanarayana, U., Ramalingam, K., Anitha, M., 2016. Metabolic and endocrine characteristics of Indian women with polycystic ovary syndrome. Int. J. Fertility Sterility 10 (1), 22.
Lone, N.M., Riaz, S., Eusaph, A.Z., Mein, C.A., Wozniak, E.L., Xenakis, T., Martineau, A. R., 2020. Genotype-independent association between vitamin D deficiency and polycystic ovarian syndrome in Lahore, Pakistan. Sci. Rep. 10 (1), 2290. https://doi.org/10.1038/s41598-020-59228-4.
Lopes, I.P., Ribeiro, V.B., Reis, R.M., Silva, R.C., de Souza, H.C.D., Kogure, G.S., da Silva Lara, L.A., 2018. Comparison of the effect of intermittent and continuous aerobic physical training on sexual function of women with polycystic ovary syndrome: randomized controlled trial. J Sex. Med. 15 (11), 1609–1619.
Manucha, W., Ritchie, B., Ferder, L., 2015. Hypertension and insulin resistance: implications of mitochondrial dysfunction. Curr. Hypertens. Rep. 17 (1), 504.
Merkin, S.S., Phy, J.L., Sites, C.K., Yang, D., 2016. Environmental determinants of polycystic ovary syndrome. Fertil. Steril. 106 (1), 16–24.
Munawar Lone, N., Babar, S., Sultan, S., Malik, S., Nazeer, K., Riaz, S., 2020. Association of the CYP17 and CYP19 gene polymorphisms in women with polycystic ovary syndrome from Punjab, Pakistan. Gynecol. Endocrinol., 1–6
Nawaz, S.K., Hasnain, S., 2009. Comparison of the optimized conditions for genotyping of ACE ID polymorphism using conventional and direct blood PCR. Afr. J. Biotechnol. 8 (20).
Ollila, M.-M.E., Kaikkonen, K., Järvelin, M.-R., Huikuri, H.V., Tapanainen, J.S., Franks, S., Morin-Papunen, L., 2018. Self-reported polycystic ovary syndrome is associated with hypertension: a northern Finland birth cohort 1966 study. J. Clin. Endocrinol. Metabolism 104 (4), 1221–1231. https://doi.org/10.1210/jc.2018-00570.
Palumbo, A., Ávila, J., Naftolin, F., 2016. The ovarian renin-angiotensin system (OVRAS) a major factor in ovarian function and disease. Reprod. Sci. 23 (12), 1644–1655.
PCOS-UK-Guidelines, 2012, 24 September 2012. Diagnosis and management of polycystic ovary syndrome, from https://www.guidelines.co.uk/womens-health/pcos-uk-guideline/236071.article.
Ramalingam, L., Menikdiwela, K., LeMieux, M., Dufour, J.M., Kaur, G., Kalupahana, N., Moustaid-Moussa, N., 2017. The renin angiotensin system, oxidative stress and mitochondrial function in obesity and insulin resistance. Biochimica et Biophysica Acta (BBA)-Mole. Basis Dis. 1863 (5), 1106–1114.
Singh, P., Agress, A., Madrigal, V., Magyar, C., Ostrzega, N., Chazenbalk, G.D., Dumesic, D.A., 2019. Massive ovarian growth in a severe insulin-resistant polycystic ovary syndrome (PCOS) woman receiving GnRH-analog. J. Clin. Endocrinol. Metabolism 104 (7), 2796–2800.
Smaism, M.F., Gatea, A.K., Ejam, Z.Y., 2016. Evaluation of insulin, insulin resistance LH, and FSHin women with polycystic ovary syndrome and diabetic mellitus type 2. Med. J. Babylon 13 (1), 73–78.
Stapinska-Syniec, A., Grabowska, K., Szpotanska-Sikorska, M., Pietrzak, B., 2018. Depression, sexual satisfaction, and other psychological issues in women with polycystic ovary syndrome. Gynecol. Endocrinol. 34 (7), 597–600.
Voloceai, V., 2017. Contemporary Profile Of The Patient With Polycystic Ovary Syndrome (PCOS). Acta Medica Transilvanica 22 (1).