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Ultrasonographic finding in women with polycystic ovary syndrome in correlation with their FSH gene polymorphism

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Abstract : PCOS is the most public reason of anovulation, sterility and hyperandrogenism in women, disturbing between five and ten percentage of women of generative age worldwide. Therefore the study focused on these cases and aimed to Ultrasonographic finding in women with polycystic ovary syndrome in correlation with their FSH gene polymorphism. "In current study thirty specimens were collected from 30 women patients (PCOS) and 30 healthy between 23 and 48 years old and examined using Ultrasonographic imaging, hormonal analysis and DNA samples from peripheral blood lymphocytes were extracted and analyzed by PCR-RFLP of FSH gene by digested with BsrI, an endonuclease that recognized the A to G transition sites at position 680 codon". Imaging of Ultrasonographic appears that all the women patients in the study gave positive results with PCOS to all cases were obtained from women patients.Then blood was collected for hormonal assay the results revealed decrease in serum LH levels with a concentration increase in follicular stimulating hormone FSH level at thewhen compared with control.The targeted fragment contains one restriction site for an endonuclease there are three possible patterns were expected (AA, GG, AG) genotype. As related with AA genotype, there are no significant differences between control group and pcos patients group, while, GA genotype frequency was significantly ($p<0.05$) higher in the control group than PCOS patients (15 *versus* 28%, respectively; X^2=4.175). In contrast, GG genotype frequency was significantly ($p<0.05$) higher in the PCOS patients group. The Allele frequencies of A and G alleles were 42% and 58% in the PCOS patients group, 52% and 48% in the control group respectively. This study concluded that when compare between hormonal and ultrasound data and molecular approach the Ultrasonographic imaging was the good and efficient methods for detection the PCOS in women patients.

Keywords: Ultrasonographic imaging, *FSH*, Polymorphism, PCOS.

Introduction

"Polycystic ovary appearance on ultrasound examination, and are diagnosed as having PCOS by the 2003Rotterdam criteria"[1]."The pathogenesis of PCOS, is poorly understood. A high incidence of alike phenotypes in family members of PCOS patients suggests that genetics may play a role"[2]."Though the genes polymorphisms that encoding sex hormones and their receptors have been investigated, results are still conflicting"[3]."FSH plays an important role in follicular growth and ovarian steroid genesis. Mutations or polymorphisms in the FSH gene can affect reproductive ability"[4]."The FSHR gene contains two important SNPs in exon ten, which are in association disequilibrium. One polymorphism is located at codon 307 in the extracellular domain of the receptor, where alanine is substituted by threonine (A307T; rs6165)". "The other polymorphism is in the intracellular domain at codon 680, where N680S; rs6166"[5,6]. "To date, few genetic studies have examined the connotation between FSHR polymorphisms and PCOS"[7,8]. "PCOS prevalence and clinical manifestations, as well as frequency of the FSH polymorphisms, can differ between ethnic and racial

groups[9], more studies should be done to authorize this conclusion". The objective of this study was to confirm the correlation between ultrasonographic findings in women with polycystic ovary syndrome with their FSH gene polymorphism.

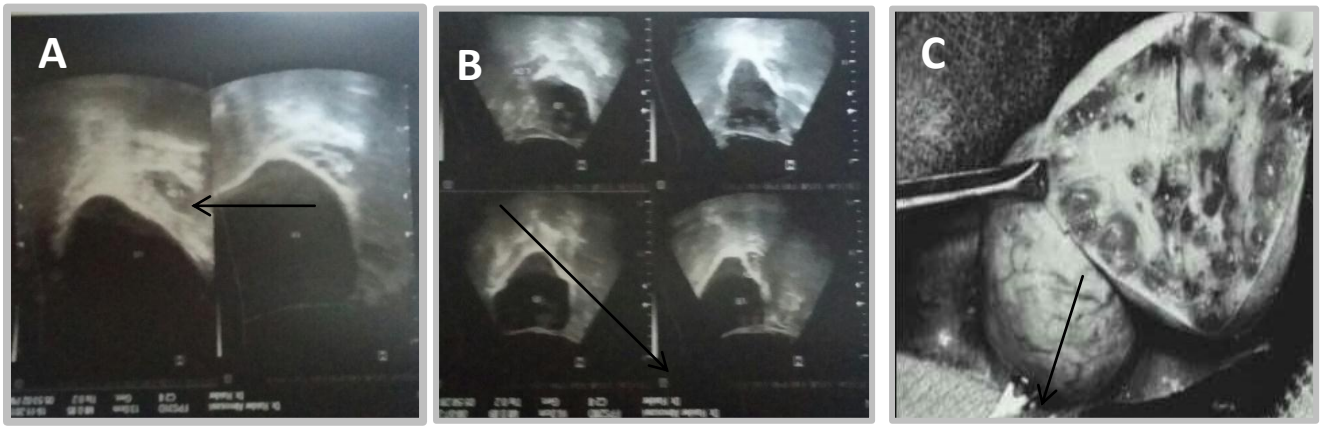
Materials and Methods

Thirty specimens were collected from 30 women patients and 30 healthy between 23 and 48 years old attending the fertility centre in Kamal Alsamarraee hospital of fertility and in *vitro* fertilization suspected to have polycystic ovary syndrome as clinically identified by a physician; in ultrasonographic analysis from the period from November 2015 to June 2016. All samples were inoculated for hormonal and molecular study. At the experiment blood samples were drawn and serum was collected by centrifugation. Follicular stimulating hormone (FSH) concentrations were measured and Luteinizing hormone (LH) in serum by ELIZA kits (Beckman-France), as presented in standard Assay[10].The results were analyzed using one-way analysis of variance (ANOVA), the level of statistic was set at $P<0.05$[11]. DNA was prepared and purified according to the genomic isolation kit provided by Geneaid Company/Taiwan. The Nanodrop system (BioDrop/UK) was used for the measurement of the concentration and purity of the DNA according to Sambrook and Russel, 2001, using 2 µl of each DNA sample. The specific primers for FSHgene and their sequences were chosen according to Valkenburg *et al.*,[12], forward 5′-TTTGTGGTCATCTGTGGCTGC-3′; reverse 5′-CAA AGGCAAGGACTGAATTATCATT-3′.PCR was performed in 25 ml; each reaction combination was heated at 10min to 95ºC. A total of 35 PCR cycles, each cycle at 0.3min at 94ºC for denaturation, 1.15 min at 72ºC for extension,0.45 min at 55ºC for annealing and a 10min final extension at 72ºC. PCR products were observed by gel electrophoresis on a 1.5% agarose gel in 1xTBE buffer stained in 0.5 mg/ml Red stain,The product PCR bands were visualized under UV light and photographed after staining the agarose gels."The PCR products were break down with BsrI(Promega,USA), an endonuclease that known the adenine to guanine transition sites". "Three possible patterns were expecteda single band of undigested products of 520bp in size, demonstrating homozygous Asn680Asn(NN); a single band of digested products of 413 basepairs, indicating homozygous Ser680Ser (SS), and threebands of 520, 413 and 107 base pairs, representing heterozygousstate for Asn680Ser". "The breakdown reaction was showed in 10 µL final volume; at 37°C for 30 min, for genotyping of considered samples, the break down fragments were electrophoresed on 3% agarose gel and stained with Red stain"."The allelic and genotype frequencies and test of HW equilibrium were done sing POPGENE software", version 1.32.

Results and discussion

Ultrasound Examination :

Ultrasonographic imaging was used to describe the ovarian appearance in women classified as having PCOS in women patients appeared that theirs different forms of PCOS in women patients included in the study as show in figure (1).



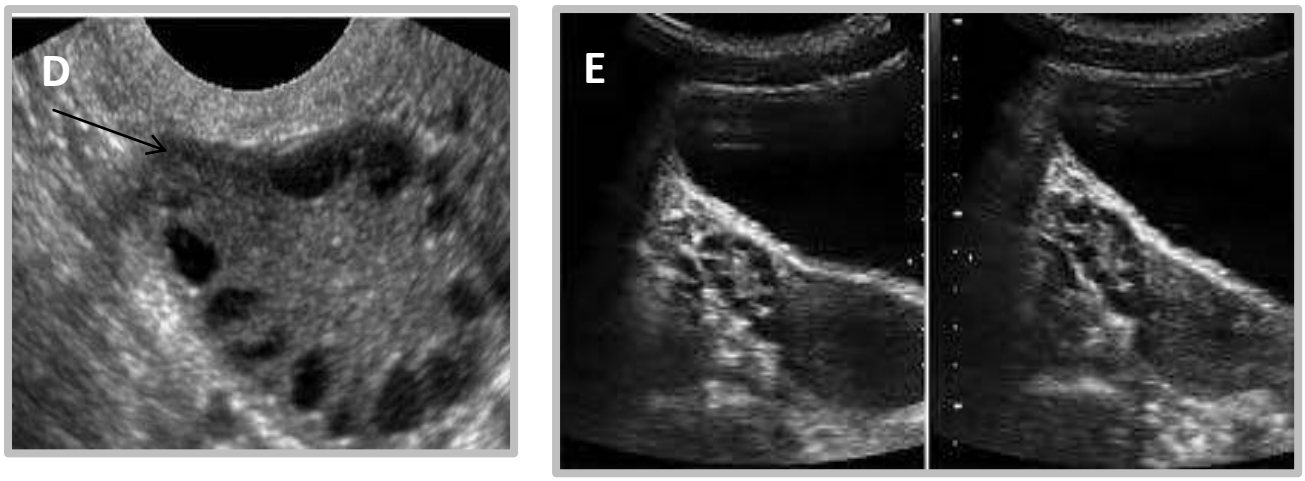


Figure (1)POS,A-Longitudinal transabdominal sonogram of an ovary. This image reveals multiple peripheral follicles.B- "Transverse endovaginal sonogram of the left ovary. This picdisplays numerous peripheral follicles and hyperechoicstroma.""Annotation that none of the follicles is larger than 1.2 cm".C- This image "reveals multiple subcapsular follicles in together ovaries; the follicles are extravisible on the left side on this image".D- "Polcysticovaries intogetherright and left ovaries, the ovarian length and width are increased as well as the ovarian area". "The follicle quantity, with a diameter mainly between Two and Five mm, is more than 8. The distribution within the ovaries is mainly peripheral. The increased and hyperechoicstroma occupies the center of the ovaries". E- "The total number of follicles per ovary or in a single cross segment was firm by summing follicle counts made in each grid section".

"The inclusion of ultrasound criteria is the important device for detection because of its apparent specificity; ultrasound features of PCOS are observed in different study apparently good results for detection[7,13]. Ultrasound has been largely superseded by transvaginal scanning to examine the patients with PCOS because of greater resolution and in many cases patient partiality, as the necessity for a filled bladder is avoided which saves time and may be more comfortable"[9,10].

"The Ultrasound method offers a more precise view of the internal structure of the ovaries, avoiding apparently homogeneous ovaries as designated with transabdominal scans, mainly in obese patients. With the transvaginal route, high-frequency probes (>6 MHz), which have an improved spatial determination but less examination depth, can be used because the ovaries are close to the vagina and the uterus and because" the presence of fatty tissue is usually less disruptive (except when very abundant)[13].

Hormonal analysis:

"Hormonal changes play important roles in the Polycystic Ovary Syndrome in women patients[14]LH and FSH are the hormones that encourage ovulation". "Both LH and FSH are secreted by the pituitary gland in the brain". "LH and FSH levels usually range between about 5-20 mlU/ml". "Most female have about equivalent quantities of LH and FSH through the initial part of their cycle". "There is a LH surge in which the volume of LH growths to about twenty five to forty mlU/ml one day before ovulation occurs". "Once the egg is released by the ovary, the LH levels goes back down[12]. PCOS cannot be diagnosed by symptoms alone". "PCOS is a very complicated endocrine disorder". "Blood tests to ration hormone heights, an ultrasound to look at your reproductive organs". "Depending on your symptoms, your physician will control precisely which tests are necessary". "Assessing hormone levels serves two major purposes. it helps to rule out any other glitches that might be causing the symptoms". "Secondly, together with an ultrasound and individual and family histories, it assistances your doctor confirm that you do have PCOS". Most often, the following hormone levels are dignified when seeing a PCOS diagnosis[9].

Table (1) Represented and summarized the "hormonal profile of patients compared with healthy in the study groups the mean value concentration of FSH mIU/ml in control 6.52mIU/ml while in patients groups reach to 16.0mIU/ml of concentrations in PCOS patients ranged between 3.5-21.5"."The results showed a general trend for FSH values to increase in patients group, this increase reached statistical significance ($P<0.05$) where the average values of serum Luteinizing hormone in control group was5.94and the average

concentrations in PCOS patients was 2.27 its ranged between 2.4 to 12.6 results appeared that LH decrease in patients when compared with the control".

Table (1): Average of Serum .SH and LH in patients with PCOS compare with healthy.

NO.	FSH, mIU/ml	LHmIU/ml
Average	16.0±0.07	2.27 ±0.05
Control		
Average	6.5±0.02	5.94±0.01

Molecular analysis:

"The genomic DNA was extracted efficiently from blood patients using a genomic DNA extraction kit to yield intact DNA with a good quality and high purity for use in PCR techniques. The concentration of the extracted DNA ranged between 903-2202 ng/µl, with a purity of 1.6-2 was obtained. *FSH* gene was successfully amplified using specific PCR primer amplification of *FSH gene* of different patients was performed in the present study to confirm the disorder in the patients included in the study compared with control. Figure (2) appeared that molecular weight of *FSH* gene was 520bp in the patients group and control"[15].

"The *FSH gene* polymorphism at position c.1890 was defined using polymerase chain reaction-restriction fragment length polymorphism PCR-RFLP with *BsrI* restriction enzyme. The targeted fragment contains one restriction site for *BsrI* enzyme and the studied *FSH gene* found within enzyme sequence (CCACAG). PCR fragments with guanine (G) at codon 680were cut into three fragments (520,107 and 413bp), whereas in fragments with adenine (A) at the same position there is no restriction site for this enzyme (520bp), therefore, the genotypes AA, GG and AG are of homozygous, heterozygous polymorphism", respectively (Figure 2).

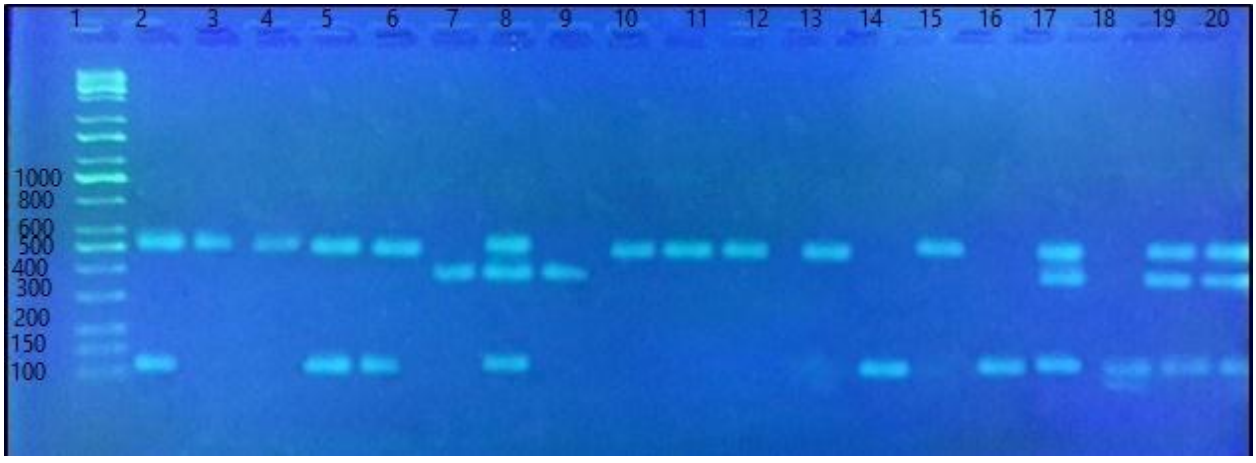


Fig.2: PCR-RFLP analysis of *FSH* digest of the PCR product at codon 680 of the *FSH gene* separated on a 2% agarose gel. DNA ladder= 100 bp, lane number (3,4, 10,11,12,13, 15) represent AA type homozygous products of 520base pairs; lane number (8, 17,19,20) three bands of 520, 413 and 107 base pairs, representing heterozygous AG; lane number (2,5,6,7,9,14,16,18) two bands of 413 and 107 base pairs, representing homozygous GG.

"The distribution of genotype and allele frequency at codon 680 of *FSH* gene presented in Table 2. As related with AA genotype, there are no significant alterations between PCOS patients group and control group, while, GA genotype frequency was significantly ($p<0.05$) greater in the PCOS patients than control group"(15*versus* 28%, respectively; X^2=4.175). In contrast, GG "genotype frequency was significantly ($p<0.05$) higher in the PCOS patients group than control group (7*versus*18%, respectively; X^2=2.434).As related with GA

and GG genotypes, there were significant differences between control and the patients groups, GA genotype frequency was significantly (*p<0.05*) less than control group than in the PCOS group (15*versus*42%, respectively; X^2=1.105).The Allele frequencies of A and G alleles were 42% and 58% in the PCOS patients group, 52% and 48% in the control group respectively".

Table (2) : Genotype of FSH (SNP Genotype/Allele).

Genotype	Patients (No.= 30)	control (No.= 30)	O.R.	Chi-square (χ^2)
AA	8 (22.00%)	7 (28.00%)	0.128	1.074 NS
GA	15 (42.00%)	17 (50.00%)	0.456	4.175 *
Chi-square (χ^2)	2.913 **	4.290 **	---	---
O.R.	0.193	0.203	---	---
AA	8 (22.00%)	7 (28.00%)	0.218	2.434 NS
GG	7(18.00%)	6 (15.00%)	0.598	2.701 *
Chi-square (χ^2)	2.349 **	0.803 NS	---	---
O.R.	0.120	0.299	---	---
GA	15 (42.00%)	17 (50.00%)	0.329	1.105 *
GG	7 (18.00%)	6 (15.00%)	0.458	2.054 *
Chi-square (χ^2)	0.543 NS	9.163 **	---	---
O.R.	0.079	1.246	---	---
Allele freq.				
A	42%	52%	---	---
G	58%	48%	---	---
*** (P<0.05), ** (P<0.01), NS: Non-significant.**				

"The frequency of A,G allele in the different population Africa, Asia, Europe, East Asia, South America appear that there's no significant difference in Africa Bantu speakers(SA001818S) where the frequency of A,G 0.440 , 0.560 respectively". While its frequency in Asia population Palestinian(SA001474Q) were 0.580 , 0.420 respectively shown table (3).At the Europe population Basque(SA001504K) the frequency of A and G Allele was 0.500 , while at the populationFrench(SA001503J) in Europe 0.600 and 0.400 respectively, at the East Asia the frequency of A and G allele in Japanese(SA002260K) was 0.630 , 0.380 respectively at the South America the frequency of A and G at the population Karitiana(SA001514L) was 0.560, 0.440 respectively.

Table (3)Geographic Region, population (Sample I D).

Geographic Region	Population (Sample UID)	Allele Symbol	
		A	G
Africa	**Bantu speakers (SA001818S)**	0.440	0.560
Asia	**Palestinian (SA001474Q)**	0.580	0.420
Europe	**Basque (SA001504K)**	0.500	0.500
Europe	**French (SA001503J)**	0.600	0.400
EastAsia	**Japanese (SA002260K)**	0.630	0.380
EastAsia	**Koreans (SA003027M)**	0.604	0.396
SouthAmerica	**Amerindians (SA001513K)**	0.850	0.150
SouthAmerica	**Karitiana (SA001514L)**	0.560	0.440

"The results on our research shows that ultrasound characteristics of the polycystic ovary has been used a good methods for detection its agreement with all reports of polycystic ovaries and PCOS published since 1970. Papers were comprised which tried to correlate features of the PCOS with specific quantitable measurements of the ovary in order to best detection the polycystic ovary"[16,17]. In present study, we found that serum FSH level in the PCOS group was increase and LH was decrease compare with the control groups, and significantly different from both. This result leads us to conclude that PCOS is apparently not a normal variant, but rather a mild phenotype of PCOS. The difference in FSH serum level compared with controls proposes an irregularity of the granulosa cells in PCOS; the excess is seemingly not triggered by the advanced number of follicles, the alteration from controls persisted after adjustment for the number of two–nine mm follicles, proposing extreme secretion of FSH per follicle. This could be a reflection of an augmented amount of granulosa cells within each follicle in the women with PCOS[18,19]. These strict selection criteria may explain the difference in our results through our selection method Ultrsonographic imaging, clinical and molecular criteria these results agreed with the results were observed in sample of women with PCOS[20].This study concluded that when compare between hormonal and ultrasound data and molecular approach the Ultrasonographic imaging was the good and efficient methods for detection the PCOS in women patients.

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