

Association of KISS1 Gene SNPs (rs12998) With Endocrine Parameters in Iraqi women Polycystic Ovary Syndrome

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Abstract

background: (PCOS) is among the most common types of gynecological hormonal abnormalities and metabolic problems that have an impact females throughout adolescence, with a prevalence of 6-10%.Hyperandrogenism, unreliable menstrual periods, and polycystic ovaries are the hallmarks of PCOS. The (HPG) axis is controlled by kisspeptins, peptides made by the KISS1 gene.

Objectives: This investigation's objective about used to be to analyze the affiliation of kiss1 Gene SNPs (rs 12998) with Endocrine Parameters in PCOS .

Methodology: A case -control find out about enrolled one hundred sixty members of which eighty female have PCOS and eighty symbolize the manage crew who had-been interestingly healthful. Patients with PCOS had been chosen patients from our health center and the teaching hospital's gynecology and obstetrics department in Tikrit. Assessment of serum (kisspeptin, estradiol ,LH, FSH and testosterone) used to be performed the use of ELISA approach and the maker company's provided methodology was followed to extract genomic DNA from whole blood. KISS1 Gene SNPs rs12998 T>C gene polymorphisms was once detected by using (T-ARMS-PCR) method.

Results: Findings indicate, the frequency of the kiss1 gene rs12998 CT and TT genotypes in PCOS-affected women increased 1.63-fold and 8.88-fold in comparison to the group of healthy women which have odds ratio (OR); 1.63 and 8.88, 95% [CI], 0.667-3.988 and 2.014-39.222; p-value 0.28 & 0.01**), respectively. The levels of Kisspeptin, LH, and testosterone also differ.

Conclusions: Our find out about suggests a greater awareness of Kisspeptin serum levels were observed in PCOS as in contrast with healthy as well, the frequency of homozygous allele (CC) used to be greater in patient 15 than in controls 3 whilst the frequency of heterozygous allele (CT) have been now not drastically distinct in PCOS fifty six much less than the controls sixty one The allelic frequency of SNP rs12998 C/T used to be detected extra regularly with significant affiliation in female with PCOS than in

Keywords: PCOS, Kisspeptin, kiss1.

INTRODUCTION

PCOS is one of the most prevalent gynecological hormonal abnormalities and metabolic problems that affect females throughout adolescence, with a prevalence of 6-10% (1-3). Hyperandrogenism, unreliable menstrual periods, and polycystic ovaries are the hallmarks of PCOS (4) these aggravate the signs of hyperandrogenemia. researchers have focused particularly on the genetics of this illness in terms of genetic factors associated to the hypothalamic-pituitary-gonad (HPG) axis (5) . KISS1, Gprotein coupled receptors receptor GPR54 a few of the HPG axis mutation that have been investigated in the past (6) . KISS1 has become one of the potential genes playing a controlling function in the female reproductive system, playing a significant good position in the generation of HPG axis gonadotropin (7) . Several SNPs in the KISS1 gene have been uncovered to regulate the HPG axis, impairing the healthy operation of the female reproductive system. These SNPs are expected to play a vital part in the etiopathogenesis of PCOS (8) . The KISS1 gene produces peptides called kisspeptins which guide HPG axis (9) Evaluation the kisspeptin and KISS1 gene polymorphism in PCOS Finding the complex etiology of PCOS may be helped by the affected ladies (10) .

METHODOLOGY

Research Design: The current investigation performed a case - control study for a collection of (160), divided to 80 PCOS-positive girls and 80 strangely healthy subjects acting as controls.

Sample size: The study was conducted on 160 women participants.

Population: 80 women with PCOS disorders and 80 women in control group.

Research setting: This study was conducted from outpatient patients and the infertility clinic at the Tikrit Gynecology and Obstetrics Teaching Hospital. in Salah Al-Din Governorate, Iraq.

Sampling Criteria

Each patient had their medical and biochemical histories, as well as their heights and weights, recorded. The self-reported questionnaire collected the sociodemographic information of the patients, including their age, BMI, and the presence of illnesses.

Methodology: Assessment of serum (kisspeptin , estradiol ,LH, FSH and testosterone) performed by the used of ELISA approach and The genetic evaluation used to be carried out at Tikrit University Department of Biology, College of Science. Each PCOS and manage woman has contributed five milliliters of peripheral blood. Blood samples were stored at 4 degrees Celsius in anticoagulant tubes. Historically, peripheral leukocyte genomic DNA was isolated using (Thermo Fisher Scientific, USA) According to the producer protocol, the isolation used to be made. NanoDrop™ evaluated the DNA's quality and integrity (Thermo Scientific, USA). The (TETRA-ARMS-PCR) method for genotyping of the SNP rs12998 T>C KISS1 gene since it is a quick and inexpensive method for SNP detection. Two external primers were used out of the four that were used(11_14) .

Results and Discussion

Endocrine parameter

According to the research, Women with PCOS differ significantly from the control group in regards of LH ,testosterone and kisspeptin and non-significant difference in E2 and FSH . The Mean \pm SD with p-value included in the table (1)

Table :1. hormonal imbalances between PCOS patients and the control group:

Hormones	Patients (80)	Control (80)	P _value
	Mean \pm SD	Mean \pm SD	
E2 (pg/ml)	40.24 \pm 18.13	38.99 \pm 12.82	0.6163
KISS (ng/ml)	1.82 \pm 0.43	1.08 \pm 0.42	\leq 0.0001
Testosterone(ng /ml)	0.97 \pm 0.25	0.32 \pm 0.08	\leq 0.0001
FSH(mIU/ml)	5.23 \pm 0.67	5.46 \pm 0.84	0.0561
LH(mIU/ml)	9.37 \pm 1.38	5.18 \pm 0.65	\leq 0.0001

A neuropeptide called kisspeptin boosts the GnRH's secretion. In order for ovulation to occur and LH to be produced, it is necessary. GnRH and LH secretion are both secreted To a different degree than women with PCOS (15) . the mean serum level of kisspeptin in patient was (1.82 \pm 0.43)and in control group was(1.08 \pm 0.42) that shows significant difference (\leq 0.0001) in a variety of studies, a rise in serum Kisspeptin levels in PCOS patients was observed, which is consistent with our findings (16, 17) . While , Other research didn't discover this difference (18) .The others Hormonal profile levels Comparison of serum hormonal FSH , LH , esradiol and testosterone conducted between PCOS patients and the control group listed in the table (1)., the mean serum level of FSH in patient was (5.23 \pm 0.67)and in control group was(5.46 \pm 0.84) that shows non-significant difference (p 0.0561)in patient in contrast to the control group. this research revealed that the serum level of LH was significant

difference ($p \leq 0.0001$) in patient group (9.37 ± 1.38) when compared with control group (5.18 ± 0.65). In this study FSH levels in PCOS women were lower, although LH levels were higher. Normally FSH level raise at the beginning of the follicular phase under the effect of GnRH, it stimulates follicles bellow (6-8mm), when these follicles grow it become under the estrogen effect. In PCOS ovaries high frequency and amplitude of GnRH were observed, this elevation mirrored by LH elevation that causes early luteinisation of granulosa cells of immature small follicles and growth arrest favoring the greater production of steroid hormone that negatively block FSH production (19). The Neuroendocrine disorders in PCOS patients affect the afferent upstream of GnRH, high androgen level participate in progesterone desensitization and alteration in the negative feedback of estrogen thus elevating LH level. Increasing androgen level in PCOS patients is the main etiological factors in a disturbing hypothalamic pituitary- axis (20). The level of estradiol in patient was statistically not significant difference ($p = 0.6163$) with mean serum level (40.24 ± 18.13) while the mean serum level (38.99 ± 12.82) in control group. In agreement with our results (21,22) found that estrogen levels in PCOS patients were significantly lower than normal women, according to the study. As opposed to that, in agreement with our result (23) demonstrated that estrogen level like androgens is higher in PCOS women. The mean serum level of testosterone was significant ($p \leq 0.0001$) in patients group (0.97 ± 0.25) when compared to control group (0.32 ± 0.08). Hyperandrogenemia is the most common hormonal abnormality in PCOS women. Hyperandrogenism can be detected biochemically by estimation of (Total testosterone, free testosterone, SHBG, androstenedion, 17-hydroxyprogesterone, Dehydro epiandrostenedion and free androgen index (24). In agreement with our result (25) also detected high free testosterone level in PCOS patients.

Genotype and allelic frequency

The distribution of alleles and genotypes for rs 12998 there are differences between the groups by PCOS and controls.

Table ; 2- lists the genotype frequencies and counts for PCOS and control groups. Cases and controls had significantly differing frequencies of the detected polymorphisms. The quantity of the (CT) was 56 in the PCOS group compared to 61 in the controls, and the p-value (0.2826) did not indicate any significant difference, while the homozygous (TT) allele was 15 in PCOS group while 3 in controls and the p-value (≤ 0.01 **) and there was a big difference. In this study, the allelic frequency of SNP rs12998 C/T turned into proven to be substantially related to PCOS compared to control, with an odd ratio of 1.613 and confidence interval (1.0370 - 2.509) (P-value = 0.03*).

Table -2-: distribution of alleles and genotypes for rs 12998 among the PCOS and control groups

Genotypes	Patients No. (80)		Control No. (80)		OR	(95% CI)	P value
	No.	%	No.	%			
CC	9	11.25	16	20	1 Ref.	-	-
CT	56	70	61	76.25	1.6321	0.6678 - 3.9886	0.2826
TT	15	25	3	3.75	8.888	2.014 - 39.222	≤ 0.01 **
CT+TT	71	88.75	64	80	1.9722	0.8150 - 4.7729	0.1320
Alleles	No.	%	No.	%	OR	(95% CI)	P value
C	74	46.25	93	58.1	1 Ref.	-	0.03 *
T	86	53.75	67	41.9	1.613	1.0370 - 2.509	

The outcomes of the current investigation showed a favorable correlation between PCOS and the kiss1 rs12998 T>C gene polymorphism. In PCOS women compared to control women, the TT-genotype frequency was considerably greater while the CT-genotype frequency was not statistically different. Positive and negative results (association) from various There were reported populations. Our results Disagree with previous reports (10). They discovered no significant variation in rs 12998 comparing PCOS and control.

Table (3): The hormonal markers and patients' alleles Among Rs 12998 in patient and control.

Biochemical parameters	Genotyping frequency		p-value	Genotyping frequency		p-value	Genotyping frequency		p-value
	PCOS Mean \pm SD CC	Control Mean \pm SD CC		PCOS Mean \pm SD CT	Control Mean \pm SD CT		PCOS Mean \pm SD TT	Control Mean \pm SD TT	
E2 (pg/ml)	47.1 \pm 23.84	37.71 \pm 13.83	0.1223	39.78 \pm 17.94	38.50 \pm 12.77	0.6594	37.85 \pm 15.13	48.91 \pm 5.71	0.1331
Kisspeptin (ng/ml)	2.22 \pm 0.33	1.14 \pm 0.45	\leq 0.0001	1.76 \pm 0.43	1.09 \pm 0.41	\leq 0.0001	1.83 \pm 0.39	0.734 \pm 0.175	\leq 0.0001
Testosterone (ng/ml)	0.92 \pm 0.24	0.33 \pm 0.09	\leq 0.0001	0.96 \pm 0.22	0.32 \pm 0.08	\leq 0.0001	1.03 \pm 0.34	0.37 \pm 0.07	0.0007
FSH (mIU/ml)	5.20 \pm 0.53	5.78 \pm 0.91	0.0956	5.18 \pm 0.68	5.40 \pm 0.81	0.1162	5.44 \pm 0.68	5.13 \pm 0.79	0.4192
LH (mIU/ml)	9.40 \pm 1.16	5.12 \pm 0.75	\leq 0.0001	9.54 \pm 1.41	5.19 \pm 0.65	\leq 0.0001	8.70 \pm 1.26	5.18 \pm 0.52	\leq 0.0001

It can be seen from the Table(3) that the LH, testosterone and Kisspeptin among allele frequency Between the PCOS groups and the control, CC is statistically significant. .. While, FSH and E2 do not statistically differ between controls and PCOS groups. , while among the CT allele showed significant difference in LH, testosterone and Kisspeptin. While, FSH and E2 is statistically not significant comparing PCOS groups with controls . and observed that in LH, testosterone, and Kisspeptin, the allele frequency CC is statistically significant comparing PCOS groups with controls. While there is statistically no difference comparing PCOS groups with controls for FSH and E2 .

Conclusion

Serum kisspeptin1 levels comparing PCOS groups with controls in this study significantly differed from each other. When comparing cases and controls, it was found that the frequencies of the identified polymorphisms varied significantly. For example, the repetition of the homozygous allele (CC) in cases was greater (15) than in controls (3) while the repetition of the heterozygous allele (CT) did not significantly contrast between cases (56) and controls (61). Women with PCOS had higher allelic frequency of SNP rs12998 C/T with substantial association than control women. The findings indicated that obese women with PCOS have a heterozygous allele for hirsutism, acne, and hair loss in the majority of instances (GC). In PCOS, the KISS1 genotyping frequency revealed a substantial relationship with LH, Kisspeptin, and testosterone. Compared to homozygous wild and heterozygous alleles, the mutant genotype's serum testosterone level was greater.

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