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The Impact of *LHR* Gene Polymorphism Rs12470652 in Women with POF and Nihh, A Case-Control Study

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Abstract

The current study was designed to investigate the impact of the missense Single Nucleotide Polymorphism (SNP), Asn291Ser (c.872A>G: rs12470652), of *LHR* gene (Luteinizing hormone receptor gene) in peripheral blood samples of Iraqi infertile women diagnosed with premature ovarian failure (POF) and normosmic idiopathic hypogonadotropic hypogonadism(niHH, patients with normal sense of smell). Following the hormonal analysis, fifty women diagnosed with premature ovarian failure and fifty women diagnosed with normosmic idiopathic hypogonadotropic hypogonadism were included as patient groups, while fifty healthy fertile women were enrolled as a control group. The blood samples were obtained from patient and control groups at Kamal Al-Samarrai Hospital in Baghdad, Iraq through March to December 2018. The genotyping of the SNP (rs12470652) was carried out by real-time polymerase chain reaction (real-time PCR) of the purified genomic DNA obtained from All-cell-pellet (ACP) of blood samples. The frequency of the *LHR* (rs12470652), p.Asn291Ser minor allele *G*, was found to be 32% healthy fertile women, 30 % of those with POF (p = 0.879), and 28% in the niHH patients (p = 0.644) Therefore, no statistically significant differences were found. This research aims to study the relationship between polymorphism in the region of *LHR* gene position: 872 A > G, (Asn291Ser), (rs12470652), and both premature ovarian failure and normosmic idiopathic hypogonadotropic hypogonadism. Therefore, the potential influence of (rs12470652) p.Asn291Ser polymorphism on both types of female infertility of an Iraqi population was evaluated. In conclusion, no impact was observed since the conducted study on a sample population of Iraqi women showed that the prevalence of *LHR* (rs12470652) polymorphisms did not significantly differ in the two infertile patients groups (POF and niHH) as compared to the healthy fertile control group.

Keywords: Luteinizing hormone receptor gene **(***LHR)*, Single nucleotide polymorphism (SNP), Premature ovarian failure (POF), normosmic idiopathic Hypogonadotropic Hypogonadism (niHH).

تأثير تعدد الأشكال الجيني لـ*LHR*، rs12470652 ، في كل من مرضى فشل المبايض المبكر و قصور الغدد التناسليةالسليم المجهول السبب ، دراسة الحالات والشواهد

سامر ثامر محمود[*1]، عبد الكريم عبد الرزاق القزاز[1]، .بشرى جواد الموسوي[2]
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الخلاصة

صُممت الدراسة الحالية للتحقق من تأثير تعدد أشكال النوكليوتيد المفرد (SNP، Asn291Ser c.872A> G: rs12470652) لجين *LHR* (الجين الخاص بتشفير المستقبل الهرموني للهرمون المحفز للأباضة) في عينات الدم الطرفية لنساء عراقيات مصابات بالعقم المتمثل في فشل المبايض المبكر (السابق لأوانه) وقصور الغدد التناسلية السليم المجهول السبب (والمعنى من السليم هنا هو النساء اللاتي لديهن حاسة الشم طبيعية). بعد التشخيص الطبي و التحليل الهرموني ، أدرجت خمسون امرأة مصابة بفشل المبايض المبكر وكذلك خمسون امرأة اخرى تم تشخيصهمبقصور الغدد التناسلية السليم مجهول السبب الى مجموعتين للمرضى وتم تسجيل خمسين امرأة صحية (ذات خصوبة عالية–ولود) حيث مثلوا مجموعة السيطرة بعد التحليل الهرموني. تم جمع عينات الدم من مستشفى كمال السامرائي ، مركز الخصوبة لعلاج العقم والأطفال الأنابيب ، وزارة الصحة ، بغداد، العراق. خلال المدة من مارس 2018 وحتى ديسمبر 2018. حيث تم إجراء التنميط الجيني لتعدد اشكال النوكليوتيد المفرد (rs12470652, SNP) بواسطة أستعمال تفاعل البلمرة المتسلسل الكمي (الوقت الحقيقي) (Real–Time PCR) و الحمض النووي الجينومي المنقى الذي تم الحصول عليه من الراسب الكلي لجميع خلايا الدم (gDNA from ACP). حيث اظهرت النتائج للجين*LHR* المتعلقة بتكرار النمط الأليلي والنمط الجيني لتعدد اشكال النوكليوتيد المفرد (rs12470652)، p.Asn291Ser ، ثبات تكرار ألايل*G* حيث وجد ان 32 % من النساء مجموعة السيطرة مقابل 30 % من النساء مجموعة فشل المبايض المبكر(الاحتمالية = 0.879) ، و 28 ٪ من مجموعة نساء قصور الغدد التناسلية السليم المجهول السبب (الاحتمالية =0.644) حيث لوحظ عدم وجود احتمالية ذات دلالة احصائية عند مقارنة المجموعتين مع السيطرة. دُرست العلاقة بين تعدد الأشكال للنوكليوتيد المفرد في منطقة الجينية والموقع الكروموسومي: 872 A> G، (Asn291Ser)، rs12470652 لجين*LHR* في كلاً من مرضى فشل المبايض المبكر و قصور الغدد التناسلية السليم المجهول السبب. لذلك ، هدفت الدراسة الحالية إلى تقييم التأثير المحتمل لـ(rs12470652 p. Asn291Ser), تعدد الأشكال الوراثية على كلا النوعين من العقمالنسائي في النساء العراقيات. حيث توصلت الدراسة التي أجريت على عينة من سكان العراق النساء، الى عدم أختلف انتشار الأشكال المتعددة *LHR*(rs12470652) بين مجموعتي المرضى المصابين بالعقم فشل المبايض المبكرو قصور الغدد التناسلية السليم المجهول السبب) مقارنةً بمجموعة السيطرة التي تمثلت بنساء سليمات (ذات خصوبة عالية – ولود).

1. Introduction

Fertility is defined as the capacity of an individual to conceive and produce offspring, while infertility is a state (disease) with a diminished capacity of an individual to conceive and bear offspring. Infertility is different than sterility as it is not an irreversible state. The up to date clinical definition of infertility states that it is the biological inability of an individual to conceive or a failure to establish a clinical pregnancy after 12 months of frequent regular unprotected sexual intercourse and without the use of contraception [1-3]. On the other side, it has been reported that two years of exposure to a sexual unprotected intercourse period would cause a 50% decrease in infertility rate in couples with delayed pregnancy [4].There are approximately 186 million people worldwide who suffer from infertility, most of them residing in developing countries [5]. However, there are differences in the prevalence of infertile women from one country to another; surveys showed that infertility affects approximately 13-15% of women around the globe, with around 30% in some developing countries [6, 7] and about 17–28% in industrialized countries [8]. This complex disorder is caused by many factors such as genetic, endocrine, developmental, or even environmental factors, whereas in some cases there is still unknown or unexplained etiology [9].

Premature Ovarian Failure (POF) sometimes referred to as premature ovarian insufficiency (POI), is a mysterious and complicated disease. The term POF is usually used to describe infertile women with an age under 40 years who present with secondary amenorrhea or primary amenorrhea along with elevated gonadotropins in the bloodstream (hypergonadotropic hypogonadism); this disease is considered as devastating when diagnosed in reproductive-aged women [10]. However, Welt [11] reported that POF is a disorder with a complicated clinical presentation and course that is poorly

defined by its name "Premature Ovarian Failure". A more scientifically accurate term for this disorder that could be appropriately modified to describe the state of the ovarian function is Primary Ovarian Insufficiency (POF).The premature depletion (Cessation of menses) of ovarian follicles before the age of 40 years, representing one of the major causes of female infertility, refers to the condition when the ovaries have lost their germinative and hormonal functions because of the exhaustion of the number of ovarian follicles prior to the typical age for physiological menopause [12, 13].

Hypogonadotropic hypogonadism (HH), or otherwise called secondary hypogonadism, is defined as a clinical syndrome resulting from gonadal failure caused by abnormal pituitary gonadotropin levels and low or even absent gonadotropins in the bloodstream. HH could have resulted from either absent or inadequate hypothalamic gonadotropin-releasing hormone (GnRH) secretion or failure of the pituitary gonadotropin secretion [14].

Female GnRH deficiency, with anosmia (absence of smell scent), is referred to as Kallmann syndrome (KS), while same deficiency with a normal sense of smell is termed as normosmic idiopathic hypogonadotropic hypogonadism (niHH). They have already been proven to be important disease models that have revealed much about the abnormalities that can be caused by the GnRH neurons as they differentiate, migrate, form networks, mature and senesce. Mutations in several genes are responsible for these highly coordinated developmental processes [15]. Several genetic variations are occurring in the genes encoding for gonadotropin hormone receptors. These polymorphisms are differently distributed among human populations with different genotype frequencies in the same population.

The alteration in these genes, as a consequence, affects sex-related reproductive features and causes diseases by modulating signal transduction, which might influences the function of the ovaries and reproductive success in general [16, 17].

FSH and LH are the main hormones involved in the final stages of follicular growth and oocyte maturation. Luteinizing hormone (LH) binds to its receptor (LHR) on the surface of theca cells and granulosa cells (GCs), resulting in progesterone, androstenedione and testosterone production, ovulation, luteinization and corpus luteum formation [18]. The LHR is a G- protein-coupled receptor (GPCR) that is mostly expressed in theca cells in the ovaries, but the expression is also presented on granulosa (GC) and cumulus cells (CC), especially in the dominant follicle reaching an ovulation stage [19-21].

The *LHR* gene was sequenced for the first time in 1990 and since then more than 300 known polymorphisms have been described for LHCGR [22, 23]. LHR is encoded by the luteinizing hormone/chorionic gonadotropin receptor (*LHCGR*) gene, which is located on chromosome 2p21 in humans [24] and responsible for the transduction of extracellular signals through the activation of G protein cascade [25, 26]. Many *LHCGR* gene SNPs have been described already, but only a few of them are considered significant because they alter the function of the receptor. The single nucleotide polymorphism rs12470652, c.872A>G, p. Asn291Ser is one of the well-studied ones, which is located in exon 10 of *LHCGR* and causes amino acid changes at positions 291 (p.Asn291Ser) near glycosylation sites of the receptor protein [27].

The SNP (rs12470652) lies near glycosylation sites of the receptor protein (codon 291), in which A is replaced by G. This leads to an amino acid change at position 680 from asparagines (A**A**T) to serine (A**G**T) [28].

Therefore, studying the mutation of LHR is of a critical importance because it might affect follicular development, ovulation and the corpus luteum function.

2. Patients, materials, and methods

2.1. Patients

The study was designed to be a case-control study. The total sample size was 150 composed of fertile and infertile women and divided as the following:

Fifty well defined premature ovarian failure (POF) patients were selected by the hormonal analysis and clinical parameters, with a basal FSH of higher than 20 IU/ml. The other fifty normosmic idiopathic hypogonadotropic hypogonadism (niHH) patients were selected by the hormonal analysis and clinical parameters with a normal sense of smell and basal FSH of less than 2 IU/ml. Both patient groups' samples were collected at Kamal Al-Samarrai Hospital, the Fertility Center for infertility treatment and IVF, Ministry of Health, Baghdad-Iraq, with average ages between 20-40 years.

The control group consisted of 50 normogonadotropic women who have naturally got pregnant and conceived at least twice. They were selected by their visit to the same hospital for a routine checkup. Patients with PCOS, Tubal factor, unexplained infertility and male factor infertility were excluded in this study, while women with an age of more than 40 years were excluded from all groups.

2.2. Hormone analysis

Serum levels of the following hormones were determined: Follicle-stimulating hormone, Luteinizing hormone, Prolactin, Estrogen, Thyroid - stimulating hormone and Anti Mullerian hormone. The concentrations were determined by using VIDAS system-kits (BioMereux, France).

2.3. Luteinizing hormone receptor gene single nucleotide polymorphism, (rs12470652)

The probe for the *LHR* gene was assessed for the SNP in exon 10 region, which included (rs12470652), (LHR, A>G). TaqMan fluorescent oligonucleotide probes and primer sequences were designed according to their reference sequence (rs12470652) in the database of NCBI (National Center for Biotechnology Information), UCSC and Primer3web version 4.1.0. The primers and probes were synthesized by Alpha DNA Ltd (Canada) and stored lyophilized at (-23˚C). The sequences of each of the probes and primers used in the allelic discrimination experiments are shown in Table- 1.

Table 1-Designed primers and probes for *LHR* SNP genotyping **(**N [Asn]) ⇒ S [Ser]) that were used in this study.

Primer/probe	Sequence (5′ →3′ direction)
Forward	CTGAAGTCCAAAAGCTCAAATGCT
Reverse	TGTGCTTTCACATTGTTTGGAAAAGT
VIC- probe	CAGACAGAGTTTTTCACATTCC
FAM- probe	CAGACAGAATTTTTCACATTCC

The Genomic DNA was extracted from All- Cell-Pellet (ACP) of peripheral blood as in the following: 1 ml of the EDTA blood was pipetted in 1.5 Eppendorf tube and centrifuged at 5000 rpm for 10 min, with 3:4 of the obtained plasma was discarded and the rest (ACP) was mixed within the reminded plasma by vortex and frozen immediately for the later genomic DNA extraction [29]. For the DNA extraction of the samples, DNA extraction Kit (WizPrepy^{TM}gDNA, Mini Kit, Korea) was used according to the manufacturer instructions, then the samples were subjected to qRT- PCR. The reaction mix was adjusted to a final volume of 25 µl and included the following: 12.5 µl TaqMan.qPCR Master mix (PROBE) / (WizPure^{TM} / Korea), 0.5µl of each primer (10 mM), 0.5µl of each probe, 5µl DNA, and 5.5µL nuclease-free water. The mix was transferred to a real-time Smart Cycler (Cepheid, Real-time PCR System, USA), which was programmed for the following optimized cycles: Enzyme activation for 5 min held at 95 ˚C, 5 cycles of each denaturation (15 sec at 95 ˚C), annealing (30 sec at 62 ˚C) extension (20 sec at 72 ˚C), and finally followed by 40 cycles of each denaturation (15 sec at 95 ˚C), annealing (30 sec at 62 ˚C) and extension (20 sec at 72 ˚C).

2.4 Statistical analysis

Statistical analysis of data was performed using SAS (Statistical Analysis System - version 9.1). One-way way ANOVA and least significant difference (LSD) post hoc test were performed to assess significant differences among means. $P< 0.05$ was considered statistically significant.

Receiver operation characteristic curve was used to identify the validity of markers as an indicator of diabetes. The markers were compared according to the area under the curve. The analysis was submitted by using MedCalcSoftware. The Pearson correlation coefficients were estimated for the parameters in each group.

MedCalc Statistical Software version 16.4.3 and SAS. (2010), SAS/STAT User's Guide for Personal Computer. Release 9.13.SAS Institute, Inc., Cary, N.C., USA,SNP statistical analysis and the Odds ratios along with the confidence intervals were calculated by using the WINPEPI computer programs for epidemiologists. The latest version of the mentioned program package is available online at http://www.brixtonhealth.com. Allele frequencies of genes were calculated by direct gene counting methods, while a significant departure from Hardy-Weinberg (H-W) equilibrium was estimated using H-W calculator for two alleles, which is available online at http://www.had2know.com.

3. Results

3.1 Hormones and clinical parameters

Patient's characteristics and hormonal analysis were measured and compared to the control group (Table-2).

Table 2-Baseline characteristics of subjects of the POF, niHH and control groups

Type of variable	POF (N=50) Mean ± STM	niHH (N=50) Mean ± STM	Control (N=50) Mean ± STM	LSD
Age (year)	31.40 ± 0.82 ^{A}	29.16± 0.73 ^{B}	30.94 ± 0.58 ^{AB}	2.01
Weight (kg)	73.70± 1.63 ^{A}	71.44± 1.48 ^{A}	69.68± 1.34 ^{A}	4.17
Height (cm)	164.50± 1.24 ^{A}	165.50± 0.97 ^{A}	164.74 ± 0.84 ^{A}	2.88
BMI (kg/m^2)	27.43± 0.56 ^{A}	26.15± 0.59 ^{AB}	25.71± 0.45 ^{B}	1.51
FSH (mIU/ml)	32.52 ±3.61 ^{A}	1.06 ±0.11 ^{C}	7.91 ±0.49 ^{B}	5.89
LH (mIU/ml)	15.82±1.80 ^{A}	1.30 ±0.12 ^{C}	5.59 ±0.90 ^{B}	3.26
FSH/LH ratio	2.39 ±0.14 ^{A}	1.04±0.14 ^{C}	1.72 ±0.08 ^{B}	0.36
PRL (ng/ml)	20.80 ±1.53 ^{B}	34.28 ±5.95 ^{A}	22.25±3.38 ^{B}	11.32
AMH (ng/ml)	0.75 ±0.16 ^{B}	0.99 ±0.12 ^{B}	2.57±0.11 ^{A}	0.37
TSH (mmol/L)	3.27±0.24 ^{B}	3.98 ±0.16 ^{A}	4.12±0.13 ^{A}	0.52
E2 (pg/ml)	37.48 ±5.85 ^{B}	52.58 ±9.06 ^{B}	87.86±9.06 ^{A}	22.74

Means with a different letter significantly different ($P \leq 0.05$)
POF-Premature ovarian failure
niHH- Normosmic idiopathic hypogonadotropic hypogonadism
STM - Standard error of the mean
LSD- Least Significant Difference

The results shown in Table-2 indicate that no significant differences were obtained in age, weight, and height of the selected groups, while significant differences in the body mass index (BMI) were observed, as follows: The body mass index (BMI) showed that subjects of all the groups were over-weighted, falling in the range of 25–29.9 kg/m^2. However, the BMI of the POF group was significantly higher than that of the control group (27.43± 0.56 *vs.* 25.71± 0.45, $p \leq 0.05$). No significant differences were obtained in BMI niHH group as compared with both the control group and POF group, with the LSD value among all three groups being 1.51.

Significant results were obtained when comparing the measured basal serum FSH of the three groups; POF group showed higher levels than the control group (32.52 ±3.61 *v*s. 7.91 ±0.49) with a probability of ≤ 0.05.

Basal FSH levels inniHH group was drastically lower than that of the control group in (1.06 ±0.11 *vs.* 7.91 ±0.49, $p \leq 0.05$) (LSD: 5.89 for the three groups).

LH level was significantly different among all groups, however, the normal value was as expected in the control group. On the other hand, comparing the POF patients with the control group showed a significant elevation (15.82±1.80 vs. 5.59 ±0.90, $p \leq 0.05$,), while the level in niHH was lower compared with the control group (1.30 ±0.12 *vs.* 5.59 ±0.90, $p \leq 0.05$,), (LSD: 3.26).

The ratio of FSH/LH hormones was calculated where POF women showed an increase compared with the control group (2.39 ±0.14 *vs.* 1.72 ±0.08, $p \leq 0.05$), (LSD: 0.36).

To detect the reserve of primordial follicles in all groups we estimated the serum Anti-Müllerian hormone in all groups. The value of AMH was low in POF and niHH patients as expected, however, comparing POF women with normal fertile women showed mean values of0.75 ±0.16 *vs.* 2.57± 0.11, $p \leq 0.05$. The mean inniHH group was also lower than the control group (0.99 ±0.12 *vs.* 2.57± 0.11, $p \leq 0.05$), while the LSD value in all three groups was 0.37.

Prolactin is also estimated in the serum of the infertile and control fertile women and demonstrated no significant differences between the POF and the control groups, while the level of this hormone was higher in niHH group compared with control group (34.28 ±5.95 *vs.* 22.25±3.38 with *p*-value ≤ 0.05), (LSD: 11.32).

Thyroid-stimulating hormone (TSH) was measured in all groups and was in the euthyroid state for all groups. However, the level of TSH in the POF group was lower than that in the control group (3.27±0.24 *vs.* 4.12±0.13) with a probability of ≤ 0.05, while the niHH group showed no significant differences in TSH compared with the control group.

Estradiol II hormone (E2) was measured in all groups as well, with the two penitent groups (POF, niHH) indicating low E2 compared with the control group (37.48 ± 5.85, 52.58 ± 9.06 and 87.86± 9.06, respectively) ($p \leq 0.05$). However, POF showed lowest E2 level than niHH group but with no significant differences.

3.1. Luteinizing hormone receptor gene single nucleotide polymorphism, (rs12470652)

The missense *LHR* SNP variants rs12470652 (A>G; located on chromosome 2 position 48-694-299) was presented with three genotypes (AA, AG and GG) and two alleles (*A* and *G*). Analysis of Hardy-Weinberg equilibrium (HWE)is shown in Table-3.

Table 3-Numbers and percentage frequencies (observed and expected) of *LHR* gene SNP (rs12470652) T/C genotypes and their Hardy-Weinberg equilibrium (HWE) in premature ovarian failure (POF) patients and normosmic idiopathic hypogonadotropic hypogonadism (niHH) patients compared with the control group.

Genotype	POF (No. = 50)				niHH (No. = 50)				Control (No. = 50)			
	Observed		**Expected**		**Observed**		**Expected**		**Observed**		**Expected**	
	No.	%	No.	%	No	%	No.	%	No.	%	No.	%
AA	28	56.0	24.5	49.0	29	58.0	25.92	51.84	26	52.0	23.12	46.24
AG	14	28.0	21	42.0	14	28.0	20.16	40.32	16	32.0	21.76	43.52
GG	8	16.0	4.5	9.0	7	14.0	3.92	7.84	8	16.0	5.12	10.24
HWE Analysis	X^2 = **5.555**; D.F. = 1; p = 0.0184 **Significant**				X^2 = **4.668**; D.F. = 1; p = 0.030 **Significant**				X^2 = **3.503**; D.F. = 1; p = 0.061 **Not Significant**			

-All results rounded to two decimal places
-LHR-Luteinizing hormone receptor
-POF-Premature ovarian failure
-niHH- Normosmic idiopathic hypogonadotropic hypogonadism

POF and niHH patients revealed genotypes that were deviated from HWE, while the control group was in a good agreement with the HWE.

There were no significant differences between genotype frequencies and allele frequencies in all studied groups. However, the results in Table- 4 show the statistical analysis of the association between genotypes and alleles of *LHR* gene SNP (rs12470652); there were no significant differences and associations between both patient groups and the control group in the studied SNP.

Table 4-Statistical analysis of the association between genotypes and alleles of *LHR* gene SNP (rs12470652) in premature ovarian failure patients and normosmicidiopathic hypogonadotropic hypogonadismpatients compared with the control group.

Type of Comparison	Genotype or Allele	Patient (No. =50)		Control (No.=50)		Odds Ratio	95% Confidence Interval.	*p*	Bonferroni Correction
		No.	%	No.	%				
POF *vs.* Control	AA	28	56.0	26	52.0	1.17	0.54 - 2.56	0.841	NS
	AG	14	28.0	16	32.0	0.83	0.35 - 1.93	0.828	NS
	GG	8	16.0	8	16.0	1.00	0.35 - 2.88	1.000	NS
	A	70	70.0	68	68.0	1.10	0.60 - 1.99	0.879	NS
	G	30	30.0	32	32.0	0.91	0.50 - 1.65	0.879	NS
niHH *vs.* Control	AA	29	58.0	26	52.0	1.27	0.58 - 2.78	0.688	NS
	AG	14	28.0	16	32.0	0.83	0.35 - 1.93	0.828	NS
	GG	7	14.0	8	16.0	0.85	0.29 - 2.54	1.000	NS
	A	72	72.0	68	68.0	1.21	0.66 - 2.21	0.644	NS
	G	28	28.0	32	32.0	0.83	0.45 - 1.51	0.644	NS

-POF-Premature ovarian failure
-niHH- Normosmic idiopathic hypogonadotropic hypogonadism
-NS- Not significant results

The prevalence of the *LHR* (rs12470652), p. Asn291Ser, minor allele *G* was found in 32% of healthy fertile women, 30 % of those with POF (p = 0.879), and 28% for niHH patients (p=0.644).
The *LHR* (rs12470652) genetic polymorphism is shown not to be a risk factor in both groups of patients of POF and niHH.

4. Discussion

Luteinizing hormone promotes the secretion of androgens by the theca cells in the ovaries, which may result in follicular maturation arrest [30]. *LH* and *LHR* genes are the most crucial genes affecting steroidogenesis with metabolic and transport pathways of sex steroids [31]. The activating and inactivating variants of the *LHR* gene can cause diseases in both sexes. Most of the missense mutations are caused by single base substitution, however, women are less affected than men by the inactivating mutation in *LHR* and the homozygous inactivating mutation of the *LHR*. If it happens in women, this mutation will lead to primary amenorrhea and infertility [32].The exon 10 of the *LHR* gene contains the coding polymorphic variant (rs12470652) that causes a missense change in amino acids from asparagine to serine at codon 291. Besides, *in vitro* experiments showed that the Asn291Ser polymorphism is associated with increased sensitivity of the receptor [27]. Another study supported this idea and stated that Asn291Ser SNP (rs12470652) in exon 10 showed an increased receptor sensitivity *in vitro*, while the prevalence between patients and controls was not differently distributed [33].

In this case-control study that was conducted on the Iraqi population, it has been found that the prevalence of *LHR* (rs12470652) polymorphisms did not differ between the two infertile patients groups (POF and niHH) as compared to the healthy fertile control group.

The frequency of the *LHR* (rs12470652), p.Asn291Ser, minor allele *G* was found in 32% of healthy fertile women, 30 % of those with POF (p = 0.879), and in 28% of the niHH patients (p = 0.644).

In one Egyptian study, the lowest LH levels and LH to FSH ratio were observed in patients with GG genotype as compared to *A* allele [34].Since we examined the distribution of this polymorphic variant in two groups of patients (POF with very high serum gonadotropin hormone levels and niHH with very low gonadotropin hormone levels), we can conclude that there was no association between hormone levels and the studied SNP.

Same results regarding the polymorphism (rs12470652) were obtained in European -Bulgarian infertile women with (PCOS), it has been found that there is no influence of (rs12470652) polymorphism on the (PCOS) manifestations in Bulgarian patients. However the (rs12470652), p. Asn291Ser minor allele G was found in (7.5%) of healthy women and (6.8%) of PCOS patients [28].
Another German study on controlled ovarian hyper stimulation (COH) with low, intermediate and high responders reported that distribution of (rs12470652) in European German population is not statistically different within low, intermediate and high responders [35].

In conclusion, The *LHR* (rs12470652) genetic polymorphism is not a risk factor in both groups of POF and niHH patients. Also, no impact was observed since the conducted study on a sample population of Iraqi women showed that the prevalence of *LHR* (rs12470652) polymorphisms did not significantly differ between the two infertile patients groups (POF and niHH) as compared to the healthy fertile control group.

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