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The Impact of Luteinizing Hormone/Chorionic Gonadotropin Hormone Receptor Gene Polymorphism rs68073206 in Men with Non-obstructive Azoospermia: A Case-control Study

Abdul-Rahim A. Ali[1], Omar F. Abdul-Rasheed[1]*, Ula Al-Kawaz[2]

[1]Department of Chemistry and Biochemistry, College of Medicine, Al-Nahrain University, Baghdad, Iraq; [2]Department of Male Infertility, High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al- Nahrain University, Baghdad, Iraq

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***Correspondence:** Dr. Omar F. Abdul-Rasheed, Department of Chemistry and Biochemistry, College of Medicine, Al-Nahrain University, Baghdad, Iraq. E-mail: dr.omarabdalrasheed@nahrainuniv.edu.iq
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Abstract

BACKGROUND: The functional consequences of the luteinizing hormone/chorionic gonadotropin hormone receptor (LHCGR) gene single nucleotide polymorphism (SNP) (rs68073206) on male infertility due to non-obstructive azoospermia (NOA) is not clear.

AIM: Examining the association of LHCGR gene rs68073206 SNPs with the incidence of NOA.

MATERIALS AND METHODS: A case–control study comprised of 70 unrelated Iraqi infertile men with NOA, divided into two groups: Group (I) who did not receive infertility treatment (33 patients with age of 31.58 ± 1.059 years) and group (II) that were receiving injectable gonadotropin treatment (37 patients with the age of 33.46 ± 1.173 years), in addition to 34 age and body mass index matched fertile men as a control group. The study population was genotyped by TaqMan assay for LHCGR (rs68073206) gene SNP. Hormonal levels were estimated by immunoassay technique while the sperm analyses were conducted in accordance with the WHO criteria.

RESULTS: A statistically significantly higher serum inhibin B levels were revealed in the infertile group (I) patients with wild GG genotype (246.445 ± 224.106 pg/ml, p = 0.0439) as compared to that of GT and TT genotypes carriers that were (85.969 ± 71.685 pg/ml) and (56.420 ± 23.988 pg/ml), respectively. Whether carrying the homozygous GG, heterozygous GT, or homozygous TT genotype, the genotyping variations of patients did not reveal a statistically significant difference in distribution compared to control individuals.

CONCLUSIONS: Genotypic prevalence of LHCGR gene rs68073206 polymorphism in infertile men with NOA did not differently distribute from that of fertile men. However, variable gonadotropin sensitivity was revealed, suggesting evidence of modulating impact on hormonal treatment outcome of male infertility.

Introduction

Infertility is a global health problem that affects up to 15% of all couples trying to conceive a child. About half of all infertility conditions caused by factors attributed to the male partner [1]. The male factor infertility etiology is complicated and not yet completely established; environment, lifestyle risk factors, and genetic causes are the probable risk factors. The genetic casualties, including; chromosomal aberrations, single-gene mutations, and polymorphisms, depict appreciable roles in provoking male factor infertility among these risk factors. Genetic abnormalities may affect approximately 15% of male infertility [2].

Azoospermia can be stated as the total lack of sperms in the sediment of an ejaculate following centrifuge analysis on at least two occasions [3], [4]. This phenomenon is observed in 1.9% of the entire population and 10–15% of men presenting with infertility [4]. Non-obstructive azoospermia (NOA) is established in approximately 15% of cases of male factor infertility [5] impacts nearly 60% of azoospermia conditions and is considered as the most severe manifestation of male infertility [6]. The NOA is caused by impaired spermatogenesis, and this state of severe germ cell failure is commonly manifested as elevated follicle-stimulating hormone (FSH) concentrations with atrophied testicles [6], [7].

The luteinizing hormone/choriogonadotropin receptor (LHCGR) receptor (also named as the luteinizing hormone receptor) through ligand stimulation by LH or by its analog hormone/chorionic gonadotropin (HCG), will induce steroidogenesis, thereby contribute to the process of spermatogenesis. In addition to its fundamental role in both males and females, fetal gender differentiation and driving reproductive-related physiology [8]. The extent of the stimulation process by gonadotropins, HCG, and LH, in terms of testosterone production in a murine Leydig cell model, was comparable [9], [10].

The LHCGR belongs to the G-protein-coupled receptors (GPCRs) class A [11]. The LHCGR gene

localized at chromosome 2p21 has consisted of 12 exons. The LHCGR extracellular domains responsible for ligand binding are encoded by Exons 1 to 10 in addition to part of exon 11. The left portion of exon 11 of the LHCGR gene is encoding for each transmembrane receptor domain consisting of seven consecutive helices and the intracellularly located C-terminal domains, specialized in transducing signaling cascades [12].

Multiple polymorphic variants were exhibited and detected in the LHCGR gene [11], [13]. Nevertheless, various polymorphisms in LHCGR were silent and unlikely to have functional significance [14]. Another study of 278 patients with maldescended testes estimated the association linking LHCGR genetic polymorphisms and the disease, and it was concluded that rs2293275 single nucleotide polymorphism (SNP) (Ser312Asn) within the LHCGR gene exon 10 was pronounced, spermatogenetic damage associated resulting in a state of NOA, as prevailed by its significant predominance amongst infertile men, whether compared to that with maldescended testicles [15]. Furthermore, it was conferred that; even though these findings of LHCGR polymorphisms by themselves, look to impart a merely marginal influence on the described phenotypes in view of male factor infertility but, by combining with effects of other polymorphisms, these genetic variants consequences could be effective [14]. It is prevailed by the forementioned literature that; the LHCGR gene polymorphisms' consequences on both the male infertility incidence and treatment effectiveness of the condition are not clearly elucidated.

The present study was designed to examine whether the presence of LHCGR gene; (rs68073206) SNPs can be associated with the incidence of NOA in infertile Iraqi men and to assess the impact of the investigated SNP genotypic patterns on modulation of the gonadal endocrine function in azoospermic infertile men.

Materials and Methods

The current study was conducted in a case- control design from February 2019 to January 2020. The protocol was approved by the Department of Chemistry and Biochemistry Scientific Committee. Seventy patients who met the requirements of the inclusion criteria had been chosen for the current study, in addition to 34 healthy normozoospermic fertile individuals, were selected to be a study control.

Male patients were recruited from The High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, Baghdad-Iraq, who could not get pregnant for at least 1 year with non-protected regular coitus. The patient's clinical condition and the sort of azoospermia had been got from their medical records, along with a specialized urologist checking and confirmation. First, two separately performed seminal fluid analyses in confirming azoospermia. The entire patients were subjected to an extensive clinical checkup, including the physical examination, clinical history, endocrinological, and seminal laboratory examinations. Obtained findings by these examinations pointed to this type of male infertility.

The inclusion criteria of the patient enrolment included; (1) a state of male factor infertility lasting for 1 year at minimum with a reproductively intact wife; (2) at least two consecutive spermiograms within 6 weeks affirming azoospermia according to the WHO criteria [3]; (3) 3 months' period at minimal free from receiving a potent androgen preparation.

Exclusion criteria were (1) any evidenced etiology of infertility post the thorough clinical laboratory investigations, including genetic disorders; (2) any confirmed infectious or immunological conditions or any major systemic illness; (3) any pathological hormonal estimates including dysfunctional thyroid or adrenal glands or overt hyperprolactinemia; (4) abnormal psychological stresses; and (5) major scrotal surgeries as vasectomy or any reported male reproductive ductal injury had been ruled out from the study.

The study was additionally attained approval by the Institutional Review Board of the College of Medicine at Al–Nahrain University in accordance with the Helsinki Committee for sample collection and performing the analyses (Annex) [16]. Enrolment in the study had have done after obtaining informed consent from all participants.

Seminal fluid analysis

The semen samples were obtained by masturbation preceded by an abstinence interval ranging from 2 to 7 days, near the laboratory for limiting the time separating the collection of semen and analysis process. The physical properties of each semen sample, including; the volume, pH value, seminal fluid color, time of liquefaction, and seminal viscosity, were investigated, then the samples were examined under the microscope to evaluate the sperms' motility levels, the vitality, concentration values, and the morphology. The collected values are then compared to the stated WHO manual's reference values [3].

Genetic analysis

The selection of candidate LHCGR gene SNPs of the NOA

The investigated SNPs in the current study were chosen relying on the ClinVar, screening OMIM, and the SNPedia. The reported SNPs described

being with a higher susceptibility for NOA regarding the association of hypothalamic-pituitary-gonadal axis deregulating potential of these SNPs with the pathophysiology of NOA (Table 1).

Extraction of genomic DNA from whole blood

QuicK-DNATM Miniprep Kit (Catalog NO. D3025) was utilized to perform purification and attain an extracted high-quality DNA from whole blood samples collected in EDTA anticoagulant-containing tubes characterized by simplified procedure design for the quick isolation processes of the entire genomic DNA from the whole blood. The obtained product was then optimized to achieve maximal recovery of samples with ultra-pure DNA without detectable RNA contaminations compatible with fresh or stored samples.

LHCGR rs68073206 SNP detection by quantitative real-time polymerase chain reaction (PCR)

The rs68073206 LHCGR genetic polymorphism genotyping had been conducted using the real-time PCR technique. The probe specific for the more frequent gene allele (wild allele G) was tagged with VIC reporter dye at 5' end, and the probe specific for the less frequent gene allele (mutated allele T) was marked with FAM reporter dye at 3' end. After reviewing the amplification plot curves, the results were verified, as shown in Figures 1 and 2.

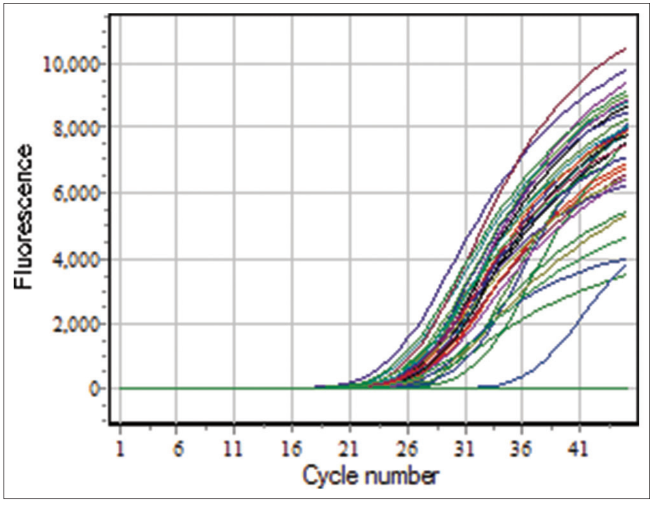

Figure 1: Amplification plot curves dependent on VIC channel fluorescence on cycle number, representing G allele (rs68073206)

All subjects were categorized as being (GG) for the homozygous wild genotype of (rs68073206) LHCGR gene, (GT) for heterozygous polymorphism, and (TT) for homozygous mutated genotype.

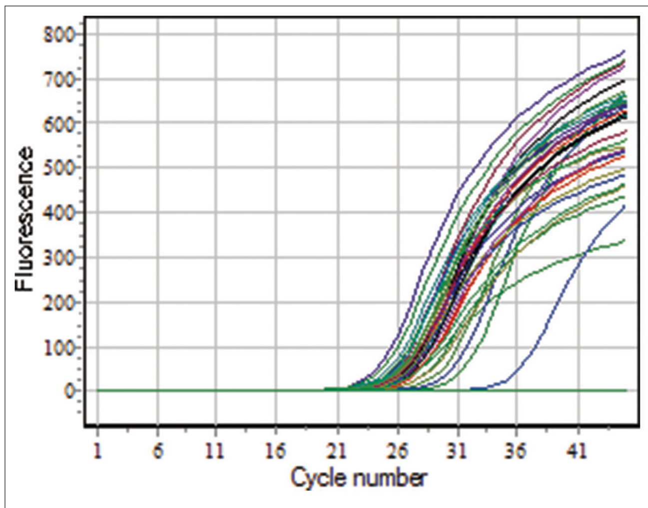

Figure 2: Amplification plot curves dependent on FAM channel fluorescence on cycle number, representing the T allele (rs68073206)

The sequence performed the PCR amplification steps; an initial step at 94°C for 5 min; then, a consecutive 30 repeated cycles comprised of steps of DNA denaturation at the temperature of 94°C for 30 s, then an annealing process at 60°C persisting for 30 s, then an elongation process at a temperature of 72°C for 1 min; with DNA final extension at 72°C for 10 min.

Hormonal assays

After venous collection of blood samples and serum prepared, levels of the following hormones were determined: Inhibin B, FSH, luteinizing hormone (LH), estradiol (E2), progesterone, free testosterone, and prolactin. The concentrations were determined in each of the two azoospermic infertile groups and the healthy fertile group. According to the manufacturer's instructions, the concentrations were determined using the ELISA technique (kits supplied by Monobind Incorporation, USA).

Statistical analysis

All statistical analyses of the study results were carried out using SPSS (version 24) software. Linear regression was used repeatedly to compare the means of clinical parameters across genotypes (To compare the means of the genotypes, linear regression was done separately). In addition, Chi-square was used to compare the effect of genotype on the responses of treatment.

Table 1: Nomenclature of the investigated gene and polymorphisms in the present study

Gene	Locus	refSNP	DNA nucleotide	Single-nucleotide variation	NCBI nucleotide reference sequence	Protein	NCBI protein reference sequence
LHCGR	2p16.3 in Homo sapiens	rs68073206	c. 536+1888T>G	g. 48948707A>C	NT_022184.16	LHCGR: Intron Variant	N/A

Results

Seventy infertile patients with NOA collected throughout the present study were divided into two groups according to their receiving infertility treatment (rFSH, rHCG or HMG injection) or not, comprised 33 azoospermic patients not received treatment yet (Group patient I) and 37 azoospermic patients receiving infertility treatment (Group patient II), as well as 34 fertile, healthy subjects as a control group as listed in Table 2.

Table 2: Mean ± SEM of age, BMI, duration of infertility of different studied groups

Groups	Age (years)	BMI (kg/m^2)	Duration of infertility (years)
Patient I	31.58 ± 1.059	26.17 ± 0.8108	5.030 ± 0.6966
Patient II	33.46 ± 1.173	28.70 ± 0.919	6.378 ± 0.505
Control	33.59 ± 1.222	26.67 ± 0.6429	–
ANOVA *P* value	0.995	0.175	0.116

*The difference is significant (p ≤ 0.05), **The difference is highly significant (p ≤ 0.01), BMI: Body mass index, Patient I: Azoospermic patients did not receive treatment, Patient II: Azoospermic patients were receiving treatment.

Anthropometric measurements and clinical characteristics of infertile patients and fertile control groups

The mean ± standard error of the mean (SEM) of age in years for the healthy control group was (33.59 ± 1.222) and for the untreated infertile patient group (patient I) with azoospermia was 31.58 ± 1.059, and for infertile patients with azoospermia (patient II) receiving treatment was 33.46 ± 1.173), respectively. No statistically significant variations (p > 0.05) were found between each of the study groups.

The mean ± SEM of the body mass indexs (BMI) in kg/m^2 for the healthy fertile control group (26.67 ± 0.6429) and azoospermic infertile patient group not receiving treatment (patient I) (26.17 ± 0.8108) and for those on treatment (patient II) (28.70 ± 0.919) respectively, there were no significant differences (p > 0.05) between them.

The mean ± SEM of the duration of infertility in years for the azoospermic infertile patient group not receiving treatment (patient I) = 5.030 ± 0.696 and for patients group on treatment (patient II) = 6.378 ± 0.505) respectively, there were no statistically significant differences detected (p > 0.05) between the patients' groups.

Results of rs68073206 SNP genotyping of LHCGR gene

Table 3 summarizes the allele frequencies and the genotypic distributions of different study subjects. The LHCGR SNP variant rs68073206 (located on chromosome 2) was presented with three genotypes (GG, GT, and TT) and two alleles (G and T).

In all patient and the control groups, statistically non-significant differences were reported in genotyping and allele frequency among individuals carrying each of the GG, GT, or TT genotypes of the LHCGR gene (rs68073206) polymorphism (Table 3).

The homozygous wild genotype (GG) of LHCGR gene (rs68073206) was revealed to be (concerning [GT] and [TT] genotypes) as following; in infertile patients group (I) with a frequency of (52%), in the patient group (II) (57%), and control group (56%), respectively. Whereas the statistical analysis revealed a non-significant variation in the distribution of the genotypes among different study groups.

To examine the statistical significance of variations in distribution frequency of the LHCGR rs68073206 G/T genotyping results, the Chi-square test was employed for the aim of investigating the odds ratios, the significance of differences of the genotypic distribution frequencies in terms of evaluating their potential risk factor for developing of azoospermia in patient groups in comparison to the healthy control group. As shown in Table 4, the genotyping variations of patients, whether carrying the homozygous GG, heterozygous GT, or homozygous TT genotype, did not reveal a statistically significant difference in distribution as compared to control individuals, so which genotype is associated with higher risk to develop the condition is did not exhibited.

Furthermore, an ANOVA test was used to stratify all of the studied serum hormonal parameters (inhibin B, LH, FSH, free testosterone E2, progesterone, and prolactin) as specified by the different genotypic variants of LHCGR gene rs68073206 SNP in numerous study groups, as revealed in Table 5.

The results revealed a statistically significant higher hormonal level of serum inhibin B in infertile patients who did not receive treatment with wild GG genotype (246.445 ± 224.106 pg/ml), and the p-value is (0.0439) as compared to that hormone levels of GT and

Table 3: Genotyping of rs68073206 LHCGR gene polymorphism with allele distribution frequency in the study groups

LHCGR rs68073206 SNP genotype frequency (%)										
Group		GG	GT	TT	G	T	Significance	Chi-squared	p-value	Total
Patient I	No.	17.00	13.00	3.00	47.00	19.00	NS	0.0507	0.975	33
	%	52	39	9	70	30				
Patient II	No.	21.00	12.00	4.00	54.00	20.00	NS	1.169	0.5573	37
	%	57	32	11	73	27				
Control	No.	19.00	10.00	5.00	48.00	20.00	NS	2.892	0.2355	34
	%	56	29	15	70	30				
Significance		NS	NS	NS	NS	NS				
p-value		0.8972	0.6751	0.7607	0.9476	0.9476				
Total		57	35	12	149	59				

LHCGR: Luteinizing hormone/Chorionic gonadotropin hormone receptor, NS: Non-significant, Patient I: Azoospermic patients did not receive treatment, Patient II: Azoospermic patients were receiving treatment.

TT genotypes carriers that were (85.969 ± 71.685 pg/ ml) and (56.420 ± 23.988 pg/ml), respectively.

Table 4: Odds ratios, p values, and confidence intervals of the LHCGR rs68073206 G/T genotype distribution frequencies in different infertile azoospermic patient groups against the control group

LHCGR rs68073206				
Groups versus control	Genotypes and Alleles	p-value	OR	95% CI
Patient I versus control	Genotype			
	GG	0.72 NS	1.192	0.455 to 3.18
	GT	0.607 NS	0.7692	0.297 to 2.17
	TT	0.38 NS	1.954	0.447 to 7.81
	Allele			
	G	0.937 NS	0.9702	0.4643 to 2.013
	T			
Patient II versus control	Genotype			
	GG	0.941 NS	0.965	0.383 to 2.44
	GT	0.260 NS	0.5401	0.198 to 1.49
	TT	0.7607 NS	1.954	0.447 to 7.81
	Allele			
	G	0.7523 NS	0.8889	0.4389 to 1.799
	T			

CI: Confidence interval, LHCGR: Luteinizing hormone/Chorionic gonadotropin hormone receptor, NS: Non-significant, OR: Odd ratio, Patient I: Azoospermic patients did not receive treatment, Patient II: Azoospermic patients were receiving treatment.

No more detected statistically significant differences among the means ± SEM of serum levels of all other studied parameters (inhibin B, LH, FSH, free testosterone E2, progesterone, and prolactin) in infertile azoospermic patient groups or the healthy individuals in control group bearing each of the GG, GT or TT genotypes of the LHCGR gene (rs68073206) polymorphism (Table 5).

Discussion

The LHCGR is a glycoprotein hormone receptor that belongs to GPCR or the seven-transmembrane receptor family [17]. The LHCGR gene that is lying within the 2p21 chromosomal location in humans involves 11 different exons. The first ten exons within the LHCGR gene are encoded for the portion of the receptor representing the extracellular domains. The remaining exon of the gene is responsible for encoding each of a little part of the extracellular receptor domains, the transmembrane domains, and the cytoplasmic C-terminal domains. It was well known that selective splicing is a commonly reported character of the glycoprotein receptors, including LHCGR. Numerous variants of the LHCGR splices were established to be originated by alternative splicing and exon skipping. Alterations in the level of the splicing variants expressions, particularly near exon 6A, result from different rs68073206 LHCGR gene polymorphs, with higher levels of expression in the testes and female granulosa cells [18]. However, the available information regarding the LHCGR variants is minimal because of their association with infertility [19].

In this case–control study that was conducted on the Iraqi population, it has been found that the genotypic prevalence of the LHCGR (rs68073206) polymorphisms had not revealed noticeable differences between the two infertile patients groups (patients with NOA receiving treatment or patient did not receive the treatment) as compared to the healthy fertile normozoospermic control group. The genotypic variations of patients, whether carrying the homozygous GG, heterozygous GT, or homozygous TT genotype, did not reveal a statistically significant difference in the distribution in comparison to the control individuals, so which genotype is associated with higher risk to develop the condition is did not exhibited (Tables 3 and 4).

Liu *et al*. and colleagues [18] were traced the LHCGR SNP (rs68073206) functional consequences approximate to the exon 6A splicing donor site throughout a study incorporated each of azoospermic, oligoasthenozoospermic, and normozoospermic individuals. The indications that prevailed in their study did not reveal an association of the rs68073206 variants and male factor infertility, to come in consistent with the results revealed by the present study. Another study of 278 patients with maldescended testes estimated the association between the LHCGR gene polymorphismic variants and the disease, and it was concluded that the LHCGR polymorphism (Ser312Asn) in the tenth exon of the LHCGR gene was significantly correlated to spermatogenetic damage owing to its higher predominance amongst the infertile men as compared to that in patients with maldescended testicles [15]. A German case study of Leydig cell hypoplasia type II phenotype with micropenis specified a compound heterozygous fashions inheritance of mutations within the LHCGR gene as a causative agent. The exon 6A localized variants resulting in LHCGR transcripts ratio alterations revealed that these explicit variants ratios

Table 5: Comparison between mean ± SD of the studied biochemical parameters with different genotypes of rs68073206 SNP of LHCGR gene in the different study groups

Groups	LHCGR rs68073206 Genotyping	Inhibin B (mg/dl)	FSH IU/ml	LH (mg/dl)	E2 (mg/dl)	Progesterone (mg/dl)	F. Testosterone (mg/dl)	Prolactin (nglml)
Patient I	GG	246.445 ± 224.106	46.758 ± 26.794	54.434 ± 23.699	152.371 ± 30.221	12.513 ± 9.267	31.149 ± 5.398	38.063 ± 7.178
	GT	85.969 ± 71.685	26.937 ± 11.128	44.271 ± 16.018	142.176 ± 40.056	15.245 ± 6.991	31.550 ± 5.658	37.909 ± 9.016
	TT	56.420 ± 23.988	35.697 ± 19.099	32.615 ± 8.451	144.711 ± 27.849	14.746 ± 9.740	34.445 ± 3.353	31.33 ± 3.215
	p-value	0.0439*	0.0825	0.1782	0.7385	0.7029	0.6258	0.3777
Patient II	GG	105.770 ± 73.177	33.793 ± 17.544	39.356 ± 8.330	148.687 ± 43.222	23.615 ± 6.862	26.552 ± 8.544	24.821 ± 11.400
	GT	78.896 ± 19.461	32.133 ± 6.362	41.250 ± 11.021	150.467 ± 65.927	21.385 ± 6.275	28.484 ± 3.700	27.201 ± 16.777
	TT	61.543 ± 32.451	41.378 ± 23.213	43.987 ± 8.652	233.392 ± 198.929	29.576 ± 5.334	26.051 ± 3.501	25.524 ± 4.889
	p-value	0.2815	0.6135	0.6217	0.1444	0.1249	0.7474	0.8891
Control	GG	418.383 ± 370.470	19.289 ± 4.029	32.591 ± 5.197	123.973 ± 24.106	17.407 ± 3.981	32.165 ± 7.708	19.996 ± 16.986
	GT	693.889 ± 386.552	24.601 ± 13.278	32.155 ± 6.422	130.470 ± 28.579	16.807 ± 2.820	36.505 ± 6.499	19.818 ± 16.810
	TT	673.109 ± 438.701	29.762 ± 15.116	33.621 ± 3.082	121.142 ± 14.773	15.335 ± 7.191	28.605 ± 3.952	14.700 ± 13.596
	p-value	0.1908	0.1068	0.9037	0.7604	0.6712	0.1524	0.8421

*The difference is significant ($p \leq 0.05$), **The difference is highly significant ($p \leq 0.01$), LHCGR: Luteinizing hormone/Chorionic gonadotropin hormone receptor, LH: Luteinizing hormone, E2: Estradiol, FSH: Follicle-stimulating hormone, Patient I: Azoospermic patients did not receive treatment, Patient II: Azoospermic patients were receiving treatment.

were pivotal for the preferable LHCGR action, whether with or without the 6A exon. It comprised a preliminary study shedding light on the exon 6A imperative character in the act of LHCGR gene supplementary regulator elements and variants that result in shifting transcriptional levels instead of protein level modulations [20]. The contradictory results concerning the relevance of a different LHCGR genotypic variants distribution to the incidence of male factor infertility revealed by these studies can be ascribed to a small sample size along with the diversity of investigated infertility conditions.

The results obtained by the current study as shown in Table 5 also revealed a statistically significant higher hormonal serum level of inhibin B in infertile azoospermic patients who did not receive treatment carrying wild GG genotype compared to that of GT and TT genotypes of LHCGR rs68073206 SNP. Several clinical investigations were asserted that the inhibin B serum levels present a valuable testicular function marker as they prevailed from the positive associations of serum inhibin B levels to the efficiency of testicular function [21], [22]. Another study noticed an exacerbation in serum levels of inhibin B after HCG administration suggesting that testosterone (produced in response to HCG) is required to stimulate inhibin B secretory potential of Sertoli cells or facilitate the stimulatory effects of FSH on Sertoli cells [23] in addition to its stimulatory effect on spermatogenesis, which reflected by inhibin B production; an important index of the spermatogenic status [24].

Furthermore, Liu *et al*.'s study showed that the *LHCGR* gene rs68073206 polymorphisms were significantly associated with serum testosterone levels among the GG genotype carriers of normozoospermic subjects [18]. Simoni and co-workers were demonstrated that the polymorphic variants of the LHCGR gene resulted in increased receptor responsiveness *in vitro* but noticed that these polymorphisms did not distribute differently between patients and controls [15].

In Liu *et al*. study at the splicing site, the SNP rs68073206 consequences were evidenced to end with alterations in the gene splicing variants related expression ratios. The exon 6A-long (the long transcript) was significantly abundant among the G/G genotype carrier cells, while these transcripts in the T/T and T/G genotypes bearing cells were revealed to be minimally detected or even unnoticeable. Furthermore, the wild-type LHCGR form expression levels were demonstrated to come with an enhanced G/G genotype tendency. Moreover, an augmented wild-type LHCGR levels give rise to increased long transcripts expressions, rendering the receptor highly susceptible to stimulations by their ligands [18]. The LHCGR variant was also found to results in an observed functional change of the LHCGR 291Ser candidate allele, the state that can be attributed to increased LHCGR sensitivity [25]. Other publications covering the genetic causes of male factor infertility were stated the association of LHCGR gene variants with low or diminished sperm counts [26], [27]. Alternatively, link the state of gonadotrophin-resistant testes and consequently male infertility to LHCGR receptor polymorphisms [28].

However, other researchers have speculated the weakness of the LHCGR SNPs contributions to a peculiar phenotype, added to the lack of a pronounced (within this gene) phenotypic markers and the absence of clear male reproductive functions related impacts [13]. Others were further proposed that various polymorphisms in LHCGR are still silent, with an excluded significance in their functional consequences [14]. Overall, it can be suggested that this enhanced serum inhibin B levels in a selected genotype carrier (GG genotype) of the studied LHCGR gene variant can be attributed to an enhanced receptor sensitivity to LH levels in Leydig cells as compared to other genotypes carriers.

Conclusion

The LHCGR gene rs68073206 polymorphisms in our populationpresented with non-obstructive azoospermia; are suggested to have atuning potential in gonadotropin receptor sensitivity and variableinfertility treatment outcome. Genotypic prevalence of LHCGR geners68073206 polymorphism in infertile men with NOA didn't differentlydistributed from that of fertile normozoospermic individuals.

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