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Single Nucleotide Polymorphism of Follicle Stimulating Hormone Receptor Gene in Iraqi Infertile Men

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Abstract

The study was directed to determine Single Nucleotide Polymorphism (rs6166 A>G) of follicle stimulating hormone receptor gene and their association as a risk factor with male infertility in Iraqi sterile patient. In the present study after seminal and serum analysis 50 patient (25 Azoospermia and 25 oligozoospermia) and 50 healthy fertile control were joined. The samples were collected from the Biotechnology Department/college of science/Baghdad university and Kamal Al-Samarie IVF Hospital, Baghdad, Iraq. through the period of two months from November 2018 to January 2019. The *SNP* (rs 6166) determination was carried out by using real-time polymerase chain reaction (qPCR) of blood samples. The difference in the mean of genotype showed a significantly different ($p<0.05$) in infertile patients group likened to corresponding means infertile control group. Survey of follicle stimulating hormone receptor gene *SNP* genotypes and allele frequencies in Azoospermia and oligozoospermia patient groups with the control group, showed that there was a significant variation in the heterozygous (AG) and homozygous mutant (GG) genotype frequencies in (rs 6166). It concluded that this *SNP* may have a role in an Azoospermia and oligozoospermia Iraqi patients complaining from idiopathic infertility.

Keywords: *Follicle stimulating hormone receptor gene, Single nucleotide polymorphisms (SNPs), Azoospermia, Oligozoospermia, Iraqi sterile patients.*

Introduction

Infertility is a disabling issue that affects public health and typically defined as the inability to conceive after a year of regular, unprotected sex 1,2. It is experienced by 10–15% of couples and is on the rise. It has previously estimated that half of these cases are due to male infertility 3. In the other hand there only a significant improvement in the diagnostic workup of infertile men 4.

The causes behind infertility are unexplained in 50% of all cases, this is described as idiopathic male infertility and frequently characterized by sperm dysfunction and spermatogenic failure 5. It is widely accepted that many genetic and environmental factors interact and are involved in the deficiency of spermatogenesis and consequent infertility 6. Genetic factors including chromosomal aberrations and single gene mutations account for 10-15% of severe male infertility, However recent research has submitted that idiopathic infertility may be produced in part by mutations or alterations in genes involved in spermatogenesis 7.

Spermatogenesis is a finely controlled process, exquisitely regulated by two gonadotropins secreted in the pituitary, namely FSH and LH 8,9.

Understanding the mechanisms of how FSH and LH regulate spermatogenesis is a very important goal in the area of male reproductive endocrinology, infertility, and contraception. FSH stimulates testicular development and spermatogenesis in the testis and folliculogenesis and steroidogenesis in the ovary 10,11. The role of FSH and its receptor (FSHR) is extensively considered in the setting of infertility and it may be relevant for impacting on ethnicity-related human reproductive achievement 12.

The FSHR is a G protein-coupled receptor with 76 kDa, part of the rhodopsin-like receptor subfamily and made of 695 amino acids 13. Located on the chromosome 2.p21, lengths more than 190 Kbases with 10 exons and 9 introns. Exons 1 to 9 encodes for the extracellular domain

allotted to ligand binding, while the largest one exon 10 that encodes part which called "hinge region", for the seven transmembrane-spanning domains and for the intracellular C-terminal tail. FSHR is expressed mainly in granulosa and Sertoli cells, in which it mediates steroid synthesis and gametogenesis. Upon ligand binding, the receptor undergoes a conformational change, leading to the simultaneous activation of multiple signaling pathways at the intracellular level 14. More than two thousand SNPs located within coding and non-coding regions of the FSHR gene were described. Two of them displaying strong relationship uncertainty were largely studied in the role of infertility: rs6165 replaces threonine by alanine, it results in a change from a polar (T) to a nonpolar, hydrophobic (A) amino acid and removes a potential O-linked glycosylation site; rs6166 exchanges asparagine for serine in the intracellular domain of the receptor, introducing a potential phosphorylation site both located in the exon 10 an amino acid alteration in the hinge region and in the intracellular domain of the protein receptor are due to these SNPs 15.

A large body of evidence available previously suggests that FSH signaling is essential for the induction and maintenance of normal spermatogenesis 16. The modulatory activity of the FSHR p.Asn680Ser SNP on fertility was recently proposed in men, in which a slight correlation between the Ser/Ser homozygous state and a lower testicular volume was found 17.

For its critical role in spermatogenesis and sperm function, polymorphisms in the FSHR gene might disturb normal spermatogenesis and affect male reproductive ability, However, little is known about the FSHR gene polymorphisms in Iraq.

In this study, we explored the possible effects SNP of the FSHR gene on male infertility and serum FSH levels in Iraq by the case-control study, which included well-defined idiopathic infertile males and fertile controls.

2.Patients, materials, and methods

2.1. patients

This study was approved by the Ethics Committee of Department of Biotechnology, College of Science, University of Baghdad, Baghdad, Iraq. Study subject was fifty Iraqi infertile male included in this study and 50 healthy fertile men from the same ethnicity without any systemic diseases were served as control. The patients were recruited from Kamal Al-Samaraie Hospital, Baghdad, Iraq. The healthy fertile men were volunteers with at least one child. With average age between (17 – 47) years old. The patient's group was divided according to the semen analysis into 25 infertile men with azoospermia (Z) and 25 infertile men with oligozoospermia (O). In addition to fifty fertile male as a control (C) to this study.

Excluded criteria included: patients with secondary infertility, abnormal karyotype, obstructive azoospermia, variococele.

2.2. FSHR gene SNPs

Primers and probes for FSH receptor gene (rs6166 A>G) were designed by Li, (2011) they provided in a lyophilized state by Macrogen Company (Korea) stored 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): primers and probes used in the study

FSHR ***(Primer for SNP Genotyping)***	
Forward	5`-GGAATGGCCACTGCTCTTCA-3`
Reverse	5`-GGGCTAAATGACTTAGAGGGACAA-`3
probe	FAM 5`-AGTCACCAaTGGTTC-`3
probe	VIC 5`-AGTCACCAgTGGTTC-`3

ReliaPrep™ Blood gDNA Miniprep System is used in this study to extract whole genomic DNA from leukocytes pellets of the blood samples according to the manufacturer instructions. Genotypes were detected by TaqMan allelic discrimination Assay on (MIC-4 Real-time PCR System, Australia).

The amplification reaction components and their final concentrations are 5µl Go Taq qPCR Master Mix (Promega/USA), 0.5µl of each primer, 0.5µl of each probe, 1.5 µl DNA, and 1.5µL nuclease-free water. The mix was transferred to a real-time thermocycler (MIC-4 Real-time PCR System, Australia).

The Real-time PCR System was programmed for optimized cycles shown in Table 2.

Table (2): The program for FSH receptor gene SNPs detection.

Steps	*°C*	*m:s*	*Cycle*
Initial Denaturation	**95**	**05:00**	**1**
Denaturation	**95**	**00:30**	100
Annealing	**60**	**00:30**	
Extension	**72**	00:30	

Statistical analysis

Data analysis was done by utilizing SPSS for Windows, version 17(SPSS

Inc. Chicago, IL, United States). Data appeared as mean ± standard deviation. Shapiro–Wilk normality test was used to determine whether the studied parameters followed a Gaussian distribution.

Variables in which the distribution of data did not conform to normality were first log transformed for analysis and then converted back to standard units for presentation. Categorical variables were analyzed by the Chi-square test. Tukey's, Dunnett, and Bonferroni Post Hoc test for multiple comparisons were applied after ANOVA tests.

Hardy-Weinberg equilibrium calculated using a web tool [18]. The difference in frequencies of genotypes and alleles between the patient groups and the control group were analyzed using the Chi-square test. Odds ratios (ORs) with a 95% confidence interval (CI) were calculated for measuring the strength of the association between the studied gene SNP and male infertility. The association degrees between variables were analyzed by Pearson correlation analysis. A two-tailed p-value less than 0.05 ($p<0.05$) was considered significant [19].

Results

3.1. *FSHR* gene SNPs

The SNP of the FSHR gene (A > G; rs6166) was presented with three genotypes (AA, AG, GG) and two alleles (A and G).

Analysis of Hardy-Weinberg equilibrium (HWE) in (Z) group and (C) revealed that the genotypes were consistent with equilibrium. And significant differences ($p <0.01$) were detected between the observed and expected genotype frequencies in both control and patients groups (table 3A).

Inspecting FSHR gene genotypes and Allele Frequencies in (Z) group and (C) group revealed that there was significant variation between these frequencies, Although a decreased frequencies of G allele (84 vs. 65%) and an increased frequencies of A allele (16 vs. 35 %) were observed in patients compared to control (Table 3 B).

In both GA and GG Polymorphisms, the odds ratio for the GA genotype was 0.09(0.01 -1.45) with p=0.025 and the odds ratio for the GG genotype was 4.12(1.26 -13.49) with p=0.021 indicating that heterozygous genotype GA and homozygous GG was a higher risk of (Z) group than the wild type AA .

Table 3A. Number and percentage frequencies of FSHR gene genotypes and their Hardy-Weinberg equilibrium (HWE) in (Z) group and (C) group.

Genotype	Z (no=25)				C (no=50)			
	Observed		Expected		Observed		Expected	
	No	%	No	%	No	%	No	%
AA	4	16.0	0.6	2.4	13	26.0	6.1	12.2
AG	0	__	6.7	26.8	9	18.0	22.8	45.6
GG	21	84.0	17.6	70.4	28	56.0	21.1	24.2
HWE Analysis	$X^{2=}$ **25** p**=0.000001** Significant				$X^{2=}$**18.264** p**= 0.000019** Significant			

Table 3B. Genotype and allele frequencies of the FSHR gene in (Z) group and (C) group.

Genotype No		Z		C		OR (95.0% CI)	P - value
		%	No	%			
AA		4	16.0	13	26.0	0.54(0.16 1.84)	0.393
AG		0	__	9	18.0	0.09(0.01-1.45)	0.025
GG		21	84.0	28	56.0	4.12(1.26-13.49)	0.021
Total		25	100%	50	100%		
Allele frequency	***G***	42	0.84	65	0.65	2.83(1.20-6.63)	0.021
	A	8	0.16	35	0.35	0.35 (0.15 -0.83)	0.021

OR, odds ratio; CI, confidence interval;

Analysis of Hardy-Weinberg equilibrium (HWE) in (O) group and (C) revealed that the genotypes were consistent with equilibrium. And significant differences ($p < 0.01$) were detected between the observed and expected genotype frequencies in both control and patients groups (table 4A).

Inspecting FSHR gene genotypes and Allele Frequencies in (O) group and (C) group revealed that there was significant variation between these frequencies, Although a decreased frequencies of G allele (84 vs. 65%) and an increased frequencies of A allele (16 vs. 35 %) were observed in patients compared to control (Table 4 B).

In both GA and GG Polymorphisms, the odds ratio for the GA genotype was 0.09(0.01 -1.45) with p=0.025 and the odds ratio for the GG genotype was 4.12(1.26 -13.49) with p=0.021 indicating that heterozygous genotype GA and homozygous GG was a higher risk of (O) group than the wild type AA .

Table 4A: Number and percentage frequencies of FSHR gene genotypes and their Hardy-Weinberg equilibrium (HWE) in (O) group and (C) group.

Genotype	**Z (no=25)**				**C (no=50)**			
	Observed		**Expected**		**Observed**		**Expected**	
	No	**%**	**No**	**%**	**No**	**%**	**No**	**%**
AA	4	16.0	0.6	2.4	13	26.0	6.1	12.2
AG	0	__	6.7	26.8	9	18.0	22.8	45.6
GG	21	84.0	17.6	70.4	28	56.0	21.1	24.2
HWE Analysis	***X^2*= 25** ***p*=0.000001** Significant				***X^2*=18.264** ***p*= 0.000019** Significant			

Table 4B: Genotype and allele frequencies of the FSHR gene in (O) group and (C) group.

Genotype No		Z		C			OR (95.0% CI)		P - value
			No		%				
		%							
AA		4	16.0	13		26.0	0.54(0.16 1.84)		0.393
AG		0	__	9		18.0	0.09(0.01-1.45)		0.025
GG		21	84.0	28		56.0	4.12(1.26-13.49)		0.021
Total		25	100%	50	100%				
Allele frequency	***G***	42	0.84	65		0.65		2.83(1.20-6.63)	0.021
	A	8	0.16	35		0.35		0.35 (0.15 -0.83)	0.021

OR, odds ratio; CI, confidence interval;

Discussion

4-1 - *FSHR* gene SNPs

Male infertility is common, especially in developing countries as a result of the complicated interaction between genetic and environmental factors [7]. Due to the importance of FSH signaling for the pubertal initiation of spermatogenesis and maintenance of quantitatively normal sperm production in adults, genetic abnormality of the FSHR, as well as FSH, would be involved in some form of male infertility, such as azoospermia or oligozoospermia. However, to date little is known about mutations or polymorphisms of the FSHR gene in Iraqi infertile men. In the present study, we established the hypothesis that the polymorphism in the FSHR gene might be the reason behind idiopathic male infertility and related to the serum FSH levels of them. The result was observed significant differences in the distribution of FSHR genotypes between infertile men and fertile males in the control group. The proportions of Asn/Asn and Ser/Ser were 16% and 84% respectively in patients groups and 26% and 56% correspondingly infertile men, while Asn/Ser genotype was not observed in the patient group through its proportion, was 18% in the control group, and there was a significant difference between the two. This result disagrees with some studies that failed to prove the difference in the prevalence of FSHR genotype infertile or infertile men[20,21]. Recent study among Iranian population found that FSHR gene polymorphism might increase the risk factor of azoospermia, wich similar to our findings [22]A2039G, and susceptibility to azoospermia in a group of Iranian azoospermic men. The association between FSH levels within the sera and A919G and A2039G alleles and genotypes were also investigated. MATERIALS AND METHODS This case control study was performed on 212 men with azoospermia (126 non-obstructive and 86 obstructive.

Despite the fact that multi unidentified factors might increase susceptibility to male infertility, the outcomes of this study showed that single nucleotide polymorphisms in FSHR gene might account as one of the susceptible factors for the etiology of idiopathic male infertility. In conclusion, the present study findings demonstrated that the genetic polymorphisms in the FSHR gene might have a role in an Azoospermia and oligozoospermia Iraqi patients complaining from idiopathic infertility. However, additional investigations are recommended to be directed on other ethnic populations to approve the results of this study.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and

scientific research ministries in Iraq

Conflict of Interest

The authors declare that they have no conflict of interest.

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