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Role of FSHR gene (rs6165) polymorphism in ovarian stimulation by exogenous FSH of Iraqi infertile women

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Abstract---Background: Exogenous FSH has been dispensed to induce follicular growth both when monofollicular development was required and when multifollicular growth was desired to generate multiple mature oocytes. Methods: 210 infertile women and 50 healthy women aged less than 34 years were recruited in present study, the infertile women were separated to three groups based on treatment response (poor ,moderate and high) responder groups. Results: The outcomes indicated that poor responder group displayed higher levels of FSH and LH also lower levels of AMH,E2,AFC. Conclusion: Polymorphism of FSHR gene(rs6165) was linked to poor response to FSH treatment of Iraqi infertile women.

Keywords---FSH, polymorphism, response.

Introduction

Infertility is described a disorder in the reproductive system described by failure to obtain a gestation after one year or more of routine sexual activity ,it is a public health issue that affects approximately eight to twelve percent of couples globally (1)infertility in females can be classified to primary infertility which happen when a female is un able to bear a child ,either because she is in capable to conceive or because in capable to carry a pregnancy to term, while secondary infertility concerning women who have previously been pregnant (2,3) .Exogenous FSH is utilized for controlled ovarian stimulation for various infertility .Despite similar

dose of exogenous FSH are used, the ovarian response varies greatly ranging from poor to hyper responder and it is critical to identify the factors that contribute to this variability, especially in the field of personalized medicine. Several parameters such as age, lowed ovarian reserve and serum level of AMH have been used as an indicator to estimate the ovarian response but FSHR are more extensively studied in connection to ovarian response in different people(4).

FSH-R is type of G-protein coupled receptor that belong to glycoprotein hormone receptor family with along extracellular domain, which consist mainly from seven transmembrane domain, three of which are short intracellular loops, three extra loops and an intracellular tail(5). On chromosome 2p21 the human FSHR gene is located and it is single copy gene that composed from 54 Kb in length. FSH is crucial for promoting follicular growth throughout the process of folliculogenesis and its action is mediated by FSHR (6). Genetic disorders caused by mutation in FSH receptor which effect on it by gain or loss of its function(7). However, many SNPs have been found in the gene of FSHR, some of these SNPs are extremely common. The most commonly SNPs are in the coding region(exon 10) of FSHR. In activating mutations in the FSHR may cause stoppage at the primary or secondary stages of follicular growth and development indicating that various degrees of FSH-FSHR action are required to induce mutation(8).

Materials and Methods

This prospective observational study was conducted on 210 Iraqi women aged 20-34 years with newly diagnosed infertility and 50 healthy women without any diseases served as control group. This study was conducted from October 2021 till July 2022. The study was approved by the Scientific and Ethical Committee in Karbala University/college of Pharmacy. All participants subjected to physical examination, vaginal ultrasonography for measurement of AFC by consultant gynecologist and basal levels of laboratory investigations (FSH, LH, Prolactin, TSH, E2, AMH) were measured in the follicular phase (2nd day of cycle). At the third day of menstrual cycle all infertile women given 75 international unit of follitropin- α (subcutaneously)(9,10) and after six days of stimulation, infertile women subjected to vaginal ultrasonography and E2 measument. Follicular development was monitored by transvaginal sonography every other day until at least one follicle reach 17 mm then hCG 10,000 IU was given as a single I.M injection to trigger ovulation. Patient group that include 210 infertile women that distributed into three groups according to treatment response (11)

- **Poor responder group:** - Include 70 infertile women which have AFC < 5 and /or AMH < 0.5.
- **Moderate responder group:** - Include 61 infertile women which have AFC 5-12 and/or AMH >2.
- **High responder group:** - Include 79 infertile women which have AFC >12 and/or AMH > 5.

Inclusion criteria

Women that included in this study must have the following criteria:

- Women with infertility has newly been diagnosed based on uterine ultrasonography and hormonal levels.
- age ≤ 34 years.
- BMI ≤ 25 .
- cycle length 27–32 days

Exclusion criteria

Previous ovarian surgery, endometriosis, endocrine and systemic disorders, male factor infertility, polycystic ovarian syndrome.

Samples Collections

Seven milliliters (7ml) of venous blood were withdrawn from all participants at second day of menses which divided into two parts ,the first part (2ml) was placed in EDTA tube for DNA extraction and the second part(5ml) was placed in plain tube for serum analysis of (FSH,LH,TSH,AMH prolactin and E2),on day nine of menstrual cycle another (2ml) of blood were drawn from all infertile women in this study for serum analysis of E2.The SNP in current study(rs6165) was elected based on national center for biotechnology information(NCBI).The DNA was extracted from blood sample based on technique of (FavorPrep™) DNA extraction kit for blood. Detection the polymorphism of FSHR SNP(rs6165) was performed by using allele specific polymerase chain reaction also called amplification refractory mutation system(ARMS-PCR).The primers were designed by usage primer-blast software and purchased from Macrogen, Korea.

Statistical Analysis

Statistical determinations were carried out by employing Statistical Package for Social Science (SPSS 25 IBM, Armonk, USA), one-sample Kolmogorov-Smirnov test used to know how the values are distributed. The data were described as the mean $\pm$ standard deviation, the differences in means of the variables between control and patient groups (poor, moderate, and high responder) were analyzed by using one-way analysis of variance (ANOVA). P value of less than 0.05 was considered as statistically significant. Genotyping results were expressed as frequency and percentage usage SPSS, allele frequency were obtained by employing Hardy-Weinberg equilibrium online calculator for all genotypes in the current study. Odds ratio (OR) and confidence interval 95% (CI-95) were employed to evaluate the association of these genotypes on the study clinical and biochemical markers as well as on the development of infertility.

Results

The demographic characteristics of women for control and patient groups (poor, moderate, high)responder were illustrated in table (1).There were no significant statistically differences regarding the age ,BMI ,as well as age of menarche in patient groups (poor, moderate, high responder infertile women) when compared to control group($p > 0.05$).

Table 1
Socio-demographic data of the control group and patient groups (Poor, Moderate, and high responder infertile women)

Mean $\pm$ SD	Control	Poor responder	Moderate responder	High responder
Number	50	70	61	79
Age(year)	25.78 $\pm$ 2.66	26.27 $\pm$ 2.95	25.39 $\pm$ 3.09	26.42 $\pm$ 3.16
Body mass index (Kg/m ²)	24.62 $\pm$ 0.74	24.13 $\pm$ 3.05	23.96 $\pm$ 2.38	24.26. $\pm$ 2.68
Age of menarche (Year)	12.76 $\pm$ 1.3	13.19 $\pm$ 1.49	12.95 $\pm$ 1.37	13.13 $\pm$ 1.1

The mean $\pm$ SD of serum FSH levels for control and patient groups (Poor, Moderate, and high responder infertile women) were 6.27 $\pm$ (1.43) mIU/mL, 9.64 $\pm$ (0.45) mIU/mL, 6.64 $\pm$ (1.48) mIU/mL, 5.67 $\pm$ (0.26) mIU/mL respectively. there were very highly significant increase ($P < 0.001$) in mean serum levels of FSH for poor responder group as compared with control group, very highly significant decrease ($P < 0.001$) occur between (moderate, and high responder infertile women) when compared to poor responder group. The mean $\pm$ SD of Antral follicle count for control and patient groups (Poor, Moderate, and high responder infertile women) were 11.7 $\pm$ 1.23, 4.14 $\pm$ 0.72, 11.16 $\pm$ 1.01, and 16.59 $\pm$ 2.16 respectively. The levels of serum AMH as mean $\pm$ SD were 3.24 $\pm$ 0.96 ng/mL, 0.63 $\pm$ 0.12 ng/mL, 2.89 $\pm$ 0.66 ng/mL, and 7.98 $\pm$ 1.46 ng/mL for control and patient groups (Poor, Moderate, and high responder infertile women) respectively.

Very high significant decrease ($P < 0.001$) in mean of AMH and AFC found in poor responder group compared with the control group, very high significant increase was found ($P < 0.001$) in mean of AMH and AFC for high and moderate responder groups compared with the poor responder group. The heterozygous genotype (CT) of FSHR gene was more abundant than (CC) and (TT) genotypes in control group with a frequency of (58,34,8%) respectively with major allele frequency of 63(63%) and minor allele frequency of 37(37%) figure (1). The TT genotype was 2.3 fold greater in poor responders than in control group and minor allele frequency in poor responder group (T allele) was more than in control, moderate and high responder groups.

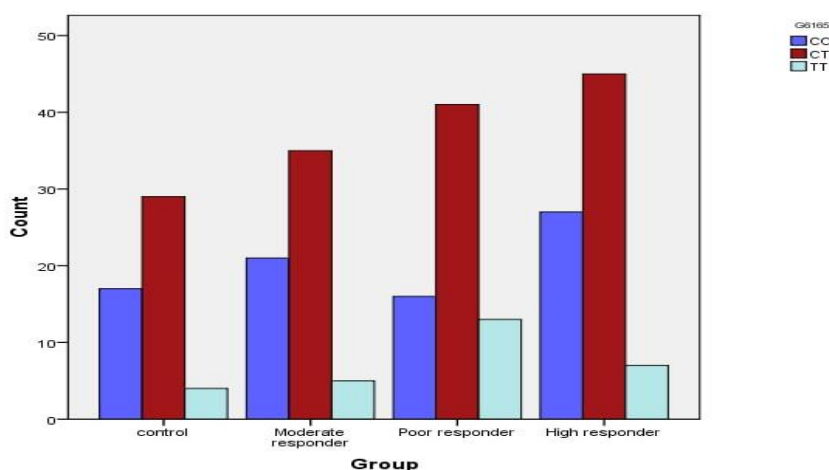


Figure 1. Genotype distribution in (rs6165) SNP among control and patient groups (poor, moderate and high responder)

The results of the present study were shown in table (4), using one-way ANOVA, the table showed hormonal parameters in each genotype of FSHR (C> T) (rs6165). In poor responder group there were very high significant increases ($P < 0.001$) in the mean of serum FSH, LH in (TT) genotype compared with the (CC and CT) genotypes in same group, while there were no significant differences ($P > 0.05$) for FSH and LH serum levels in moderate and high responder groups between all genotypes (CC, CT, and TT).

Table 1

Comparison between mean $\pm$ SD of the studied hormonal parameters with different genotypes of (rs6165) SNP of FSHR gene in the study groups

	Genotype	Poor responder	Moderate responder	High responder
Number		70	61	79
FSH (mIU/mL)	CC	8.38 $\pm$ 0.35	6.3 $\pm$ 0.64	5.36 $\pm$ 0.15
	CT	8.59 $\pm$ 0.44 c NS	7.34 $\pm$ 1.01 c NS	5.83 $\pm$ 0.1 c NS
	TT	11.96 $\pm$ 0.38 c Δ ***, d Δ ***	7.2 $\pm$ 0.53 c NS, d NS	5.91 $\pm$ 0.24 c NS, d NS
LH (mIU/mL)	CC	7.58 $\pm$ 0.59	4.9 $\pm$ 1.27	6.99 $\pm$ 0.23
	CT	7.25 $\pm$ 1.14 c NS	5.56 $\pm$ 0.55 c NS	7.59 $\pm$ 0.2 c NS
	TT	9.63 $\pm$ 0.72 c Δ **, d Δ **	5.41 $\pm$ 0.81 c NS, d NS	7.66 $\pm$ 0.23 c NS, d NS

The mean $\pm$ standard deviation (SD) of serum AMH level for (CC, CT, and TT) genotypes for poor responder group were 0.7 ($\pm$ 0.09) mIU/mL, 0.73 ($\pm$ 0.04) mIU/mL, 0.48 ($\pm$ 0.1) mIU/mL, respectively, also mean $\pm$ (SD) of AFC for (CC, CT, and TT) genotypes in poor responder group were 5.3 ($\pm$ 0.25), 4.14($\pm$ 0.72), 3.02

(± 0.42) respectively. The levels of AMH and AFC mean in (TT) genotype for poor responder group show high significant decrease ($P < 0.01$) as compared with (CC) genotype, while there was significant decrease ($P < 0.05$) in (TT) genotype in comparison to (CT) genotype. The mean $\pm$ standard deviation (SD) of serum E2 levels after treatment with FSH in (CC, CT, and TT) genotypes for poor responder group were 83.74 ± 5.31 (pg/mL), 82.83 ± 4.64 (pg/mL), 74.54 ± 4.11 (pg/mL), respectively. The size of graafian follicle levels in (mm) after treatment with FSH for all groups, mean $\pm$ (SD) for poor responder group in (CC, CT, and TT) genotypes were 11.37 ± 1.86 , 10.94 ± 2.4 , 6.64 ± 2.16 and for poor responder group mean $\pm$ standard deviation (SD) levels for number of graafian follicle to (CC, CT, and TT) genotypes were $1.21 (\pm 0.25)$, $1.17 (\pm 0.72)$, $0.75 (\pm 0.53)$, subsequently. By using ANOVA test the results exhibit insignificant difference ($P > 0.05$) in mean serum E2 levels, size and number of graafian follicle after treatment for (CT) genotype compared with the (CC) genotype in poor responder group, although there was very high significant decrease ($P < 0.001$) when comparing TT genotype with (CC and CT) genotypes in the same group.

Discussion

There were significant increment of FSH level in poor responder group with TT genotype rather than in the CC genotype and in the CT genotype in the same group, while there were no significant difference in FSH level between the three genotypes (CC, CT and TT) in moderate and high responder groups, basal FSH serum level was used to evaluate the ovarian reserve, in which high serum FSH level usually predict a high ovarian threshold to exogenous FSH responses, these finding suggested that poor responder group with TT genotype were less responsive to FSH, also the analysis of allele frequency revealed that poor responders had a greater frequency of the allele T so the T allele is associated, with poor ovarian response to FSH treatment. The presence of polymorphism of FSHR(rs6165) in poor responder group were related to elevated level of FSH and decrease sensitivity to it, whereas there were no association between FSHR genotypes with ovarian response in moderate and high responder group. The findings of present study were similar to studies reported by many researches(12-14) which show that the infertile women carriers mutant variant in poor responder group have high basal FSH level and poor response to exogenous FSH. The results of the current study was disagree with Camila M. which conclude that polymorphisms of Thr307Ala(rs6165) did not affect the FSH and estradiol serum levels and not to be associated with ovarian response(15)

In the present study, after administration of FSH dose for stimulation of the ovaries resulting in significantly low serum levels of estradiol in infertile women which carried TT genotype in poor responder group compared to women with the CC and CT genotypes. FSH stimulated granulosa cells produce E2. Therefore, impaired FSHR activity by rs6165 leads to poor proliferation and differentiation of granulosa cells and reduced production of E2 levels(16,17). These results suggested that the polymorphism in FSHR (rs6165) was associated with poor response to FSH and the T allele may be responsible for decrease sensitivity of FSH receptor to FSH. The current study was in line with many studies such as(18-20) which realized that infertile women with homozygous mutant genotypes for both study SNPs (rs6166 and rs6165) showed lower E2 level and fewer mature

oocyte than other genotypes, but the data of present study was disagree with Camila M. that showed FSH and estradiol serum levels were not associated with polymorphisms of FSHR (rs6165)(21).

In the present study the size and number of graafian follicles was significantly lower in FSHR (rs6165) TT genotype carriers in poor responder group than in CC and CT carriers. Decrease the action of FSH reflected by decrease size and number of graafian follicles while there were no significant difference among CC, CT and TT carriers in moderate and high responder groups, that mean no association of FSHR polymorphisms with genotypes of these groups. These results were in agreement with Mohammad H. that showed the lower ovarian response in poor responder group with T/T genotype including high basal FSH and lower number of mature oocytes(22). Islam A. and Radia B. showed that SNPs of FSH receptor impact on number of graafian follicles and this effect on the response to ovarian stimulation in infertile women(23,12).

Conclusion

Polymorphism in FSHR gene(rs6165) was associated with poor ovarian response to exogenous FSH.

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