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Study the correlation between *androgen receptor gene* polymorphism(rs1337082) with male infertility in samples of Iraqi who abuse anabolic steroids

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ABSTRACT: Polymorphisms that increase risk factor for male infertility is one of the most studied infertility genetic factor however polymorphisms within pre-miRNA sequences have not extensively documented. This study aimed to highlighted association between abuse anabolic steroids and male infertility by evaluate the genotyping and allele frequency of *androgen receptor* gene of rs1337082, and found the relationship of this SNP and some serum hormones levelsThe samples for this study were obtained from the Kamal Al- Samara'ay IVF Hospital, Ministry of Health in Baghdad, Iraq. The study was conducted at the Institute of Genetic Engineering and Biotechnology, University of Baghdad. The study's allocated time was extended from November 2022 to April 2023. Genotyping of rs1337082was done by HRM technology. The hormonal assays were done in another related paper. The results of distribution genotype and allele frequency of androgen receptor gene (rs1337082) polymorphisms in patients and controls show the frequency of wild AA genotype was lower in groups of patients than in control (75.00% *versus* 92.00% respectively. In contrast, the frequency of Mutant GG genotype was higher in patients when in comparison with control (8.33% *versus* 0.00%, respectively). The results show the frequency of A and G alleles in control and patients groups. Allele frequency revealed that A is more frequent in patient's groups while G is more frequent in control groups than A allele so distribution of these two alleles revealed that mutant allele is the risk allele. The conclusion of this study found Males with GG genotype are more susceptible to by thirteen times and they are more susceptible to by nine times while they are five times more likely to diagnosed with infertility, and the relationship between rs1337082 SNP and seminal fluid of patients group polymorphisms were seem not to be significant in all parameters but a possible contribution to male infertility.

Introduction

Infertility defined as genital system ailment makes the body incapable of fulfill fundamental function of reproductive system [1]. Global annual statistics revealed that approximately 60-80 million of couples facing infertility annually [2]. Male infertility account approximately 50% among all infertility cases. Up to half of male infertility cases referred to unknown causes [3]. Anabolic steroids, commonly known as androgenic steroids, are synthetic testosterone derivatives that are denoted as (AAS) [4]. The usage of anabolic steroids, both normal and illegal, is becoming more prevalent [5]. AAS is frequently used to enhance appearance, performance, and the development of muscles and body fat. Synthetic steroid hormones known as anabolic-androgenic steroids have both androgenic and anabolic effects. Because they include a steroid ring, they are classified as steroids [6,7].

The formation and maintenance of the male phenotypic and spermatogenesis depend on androgens and a functional androgen receptor (AR). This is supported by the fact that variations in the AR gene result in a range of androgen insensitive abnormalities, from total feminization to phenotypically infertile males [8].

Materials and Methods

A case-control design was used when creating the study. There were 100 samples of semen, 50 of which were from infertile men who take anabolic steroids, and 50 from healthy, normal controls. samples were taken from men who had not engaged in sexual activity for three to five days. Semen samples were collected in plastic, sterile containers, incubated under proper conditions for an appropriate amount of time, and then examined macroscopically and microscopically in accordance with WHO guidelines (2010) [9]. Using the Wizard genomic DNA purification kit from Promega, genomic DNA was isolated from the entire blood of men who were both fertile and infertile. The targeted fragments were then amplified using PCR from the retrieved DNA. After checking the size and specificity of the product with the Graphic software provided on the NCBI website, specific primers were employed for the gene. The Alpha DNA Company provided each primer as a lyophilized product in a range of picomole quantities. Table 1 contains a list of these primers' sequences.

Table (1): Primers of polymorphism genotyping

Prime Name	Primer Sequence for rs1337082	Product Bp	C
Common Forward	TTTGTGATCTTGAACAATTATTTAACCTT	29	60
A-RW	TATATTGATGAGAAAAACCAAGGTTTCG**A**	29	60
G-RW	TGCATTGATGAGAAAAACCAAGGTTTCG**G**	29	60

The PCR conditions for SNPs fragment amplification were as follows: 35 cycles of denaturation at 95 C for 15 minutes, annealing at 60 C for 30 seconds, and extension at 72 C for 30 seconds.

Sperm production is a hormonal controlled process, hence it is necessary to evaluate the hormonal profile of this study participants, The Hormonal assay (FSH, LH, Testosterone, Estrogen and Prolactin) was performed by using cobas e 411 device. were done in another related paper [10].

The Statistical Analysis System- SAS (21) application was used to evaluate the impact of various variables on research parameters. In this investigation, the Chi-square test was utilized to compare percentages and odds ratio estimates. [11].

Results and Discussion

The present study included 100 subjects equally divided into two groups, Iraqi Patients with abuse anabolic steroids (AAS) of male infertility and normal healthy controls, semen sample were collected from both groups for seminal fluid analysis, and blood samples were collected from both groups for molecular study. The age of patients and healthy control was ranged between 21 to 55 years. The seminal fluid analysis (SFA) parameters were evaluated in semen samples of abuse anabolic steroids and healthy controls.

Seminal fluid parameters

The results of semen analysis for the fertile (control) and infertile males, it was found that there are significant differences in the semen count, motility, sluggish, dead, active, normal, abnormal and volume in infertile male and control group as shown in table 2.

Table (2): Comparison between patients and control groups in Seminal fluid

Group	Mean ± SE							
	Count million	**Motility %**	**Sluggish %**	**Dead %**	**Active %**	**Normal %**	**Abnormal %**	**Volume (ml)**
Patients	2.94 ±0.14	4.02 ±0.20	82.50 ±2.48	84.50 ±2.55	7.26 ±0.38	8.08 ±0.39	85.08 ±2.56	2.06 ±0.07
Control	82.60 ±0.97	83.00 ±0.57	7.02 ±0.32	7.18 ±0.32	83.90 ±0.64	84.90 ±0.58	7.82 ±0.43	3.05 ±0.09
T-test	1.948 **	1.202 **	4.970 **	5.106 **	1.481 **	1.390 **	5.159 **	0.238 **
P-value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
** (P≤0.01).								

Rapid progressive motility percentage was in the control group significantly (P≤0.01) higher than in the patient group (83.00 ±0.57 *versus* 4.02 ±0.20 % respectively). Sluggish progressive motility percentage was in the control group significantly (P≤0.01) higher than patients group (7.02 ±0.32 *versus* 82.50 ±2.48 was respectively).

The non-progressive motility (Dead) percentage was in the patient group significantly (P≤0.01) higher than in the control group (7.18 ±0.32, *versus* 84.50 ±2.55 respectively).

The Active sperms percentage was in the control group significantly (P≤0.01) higher than in the patients group (83.90 ±0.64 *versus* 7.26 ±0.38, respectively). Normal sperms percentage was in the control group significantly (P≤0.01) higher than in the patient group (84.90 ±0.58 *versus* 8.08 ±0.39 % respectively). Abnormal sperms percentage was in the patient group significantly (P≤0.01) higher than in the control group (85.08 ±2.56 *versus* 7.82 ±0.43 % respectively). Using the Mann-Whitney U test,

the sperm count of the AAS users group shows a significant difference, and the steroid user group shows a highly significant reduction in sperm count compared to the control group (P-value =0.00); similar data were obtained from another study conducted by Hashim and Almukhtar (2021), who agree with the findings related to sperm counts but disagree with the findings related to sperm motility and morphology [12]. However, another research on the impact of Nandrolone on sperm quality done by Sharef et al., (2017) agrees with all of the present study results about sperm count, motility, and morphology. [13]. The use of AAS by bodybuilders directly affects sperm counts; the decline in sperm counts can be attributed to the hormonal imbalance brought on by AAS abuse; this suggests that AAS users' testes are negatively impacted by this downregulation of sperm production, in contrast to the control group, where sperm counts are unaffected, indicating that weightlifting without the use of AAS has little to no impact on spermatogenesis, even if taking protein supplements as the study conducted by Tøttenborg *et al*., (2020) [14,15].

Molecular study

genotype and allele frequency of *androgen receptor gene* (rs1337082) polymorphisms in patients and controls

The results of genotypes and alleles frequencies of rs1337082 SNP in controls versus Iraqi Patients with abuse anabolic steroids are presented in Table (3).

Table (3) show that the frequency of wild AA genotype was lower in groups of patients than in control (75.00% *versus* 92.00% respectively. In contrast, the frequency of Mutant GG genotype was higher in patients when in comparison with control (8.33% *versus* 0.00%, respectively).

Table (3): Genotype distribution and allele frequency of rs1337082

Genotype / rs1337082	Patients No. (%)	Control No. (%)	Chi-Square (χ^2)	P-value	O.R. (C.I.)
AA	36 (75.00%)	46 (92.00%)	1.219 NS	0.269	Ref. =1
AG	8 (16.67%)	4 (8.00%)	1.333 NS	0.248	0.502 (0.24-1.16)
GG	4 (8.33)	0 (0.00%)	2.709 NS	0.154	0.641 (0.37-1.42)
Total	48	50			
Allele	**Frequency**				
A	80 (0.83)	96 (0.96)	P-value = 0.227 NS		
G	16 (0.17)	4 (0.04)	P-value = 0.0073 **		
** (P≤0.01), NS: Non-Significant.					

The results show the frequency of A and G alleles in control and patients groups. Allele frequency revealed that A is more frequent in patient's groups while G is more frequent in control groups than A allele so distribution of these two alleles revealed that

mutant allele is the risk allele. Males with GG genotype are more susceptible to by thirteen times and they are more susceptible to by nine times while they are five times more likely to diagnosed with infertility.

Researchers looked examined six SNPs in the *androgen receptor gene* in Chilean patients with primary spermatogenic failure. One of these SNPs was the rs1337082 SNP. They (Ibid) discovered no impact of this SNP, either individually or in the context of a haplotype, on the frequency of primary spermatogenic failure. The findings of this study on the rs1337082 SNP are consistent with results. [16,17].

Impact of polymorphism on semen parameters

The relationship between rs1337082 SNP and seminal fluid of patients group polymorphisms were explained in table 4 seem not to be significant in all parameters although it cannot be ruled out that additional genetic and environmental variables, whether present alone or in combination, may contribute to male infertility [18].

Table (4): Relationship between rs1337082 SNP and Seminal fluid of patients group

Genotype / rs1337082	Mean ± SE							
	Count x 10^6	Motility %	sluggish %	Dead %	Activity %	Normal %	Abnormal %	Volume
AA	2.97 ±0.16	4.25 ±0.22	83.33 ±2.48	85.28 ±2.55	7.47 ±0.43	8.17 ±0.43	85.52 ±2.56	2.09 ±0.09
AG	2.62 ±0.46	3.12 ±0.55	74.37 ±10.70	77.50 ±11.14	6.37 ±1.18	8.12 ±1.31	78.12 ±11.21	1.94 ±0.11
GG	2.75 ±0.47	3.25 ±0.62	91.25 ±2.39	93.75 ±1.25	6.25 ±1.25	6.25 ±1.25	92.50 ±2.50	1.88 ±0.24
LSD value	0.807 NS	1.084 NS	1.33 NS	13.89 NS	2.140 NS	2.22 NS	14.38 NS	0.404 NS
P-value	0.578	0.136	0.197	0.232	0.537	0.459	0.351	0.618
NS: Non-Significant.								

***Androgen receptor gene* polymorphism and Hormones**

Table (5) explained the relationship between rs1337082 SNP and Hormones level of patients group, the results show significant positive correlation between (Testosterone and Estrogen) hormones and *Androgen receptor* gene and negative correlation with Prolactin, LH and FSH. The study of Badran *et al*., 2009 found the length of the CAG repeat and the level of blood testosterone were shown to be significantly correlated. [19]. The Bustamante study reported that the less common haplotypes with higher follicle-stimulating hormone blood levels were more likely to harbor the CAG 21 allele, which was previously associated with an elevated risk of idiopathic spermatogenic impairment [16,20].

Table (5): Relationship between rs1337082 SNP and Hormones level of patients group

Genotype / rs1337082	**Mean ± SE**				
	FSH	**LH**	**Testosterone**	**Estrogen**	**Prolactin**
AA	1.301 ±0.14	0.601 ±0.15	13.02 ±1.10 ab	105.52 ±8.44 ab	16.53 ±3.28
AG	1.296 ±0.36	0.790 ±0.39	10.26 ±2.51 b	85.67 ±9.33 b	10.73 ±3.30
GG	0.820 ±0.15	0.187 ±0.03	17.62 ±1.41 a	139.87 ±37.91 a	24.97 ±17.32
LSD value	0.891 NS	0.954 NS	6.870 *	52.419 *	16.891 NS
P-value	0.596	0.562	0.0493	0.044	0.487
Means having with the different letters in same column differed significantly. * (P≤0.05), NS: Non-Significant.					

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