International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.10 No.12, pp 233-240, 2017

Evaluating the Effect and Distribution of Exon-1 Integrity for Androgen Receptor Gene in Idiopathic Male Infertility Groups in Iraq

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Abstract : Infertility is a major health problem which affects approximately 22% of married couples in reproductive age. Androgens (testosterone and dihydrotestosterone) from another side are essential for male fertility and the maintenance of spermatogenesis, and to determine the expression of male phenotype, and their actions are mediated by single androgen receptor (AR).

So any mutation that disrupts (AR) functions completely or partially results in androgen insensitivity syndrome with impaired spermatogenesis and even XY genotype.

In the present study, male patients with infertility divided as (non-obstructive azoospermia, oligo and oligoasthenozoospermia) were studied in order to investigate the molecular genetics and molecular analysis for androgen receptor gene alteration, as a reason of male infertility in Iraq.

In the present study 100 patients (39 azoospermia, 16 Oligo and 45 oligoasthenozoospermia) were examined, and 30 normal men were subjected for detection of androgen receptor gene alteration using molecular analysis by polymerase chain reaction (PCR) for exons (1) of androgen receptor gene.

The results show deleted exons (1) as detection by PCR in the groups of infertile men but control group.

The Androgen receptor (AR) gene deletion was considered in most infertile groups as compared with control group, and in exons (1) the highest percentage of deletion was registered in oligoasthenozoospermic patients 40% from wild exon in a highly significant differences ($P<0.01$), but azoospermic patients wasn't deleted in exon (1).

The patients with deleted exon 1, registered decrease in sperm (morphology, motility, progressive motility) and grades (A and B) of sperm motility, while grade (D) was increased as compared with the wild exon (1) in a highly significant differences ($P<0.01$).

The results demonstrated the necessity of the exons (1) presence and integrity of the AR function and spermatogenesis process.

Keywords : Infertility, Androgen receptor, oligoasthenozoospermia, exons (1), spermatogenesis.

I. Introduction

Infertility is defined by the failure to achieve a successful pregnancy after 12 months or more of appropriate, timed unprotected intercourse or therapeutic donor insemination. Earlier evaluation and treatment

may be justified based on medical history and physical findings and is warranted after 6 months for women over age 35 years[1].

Infertility is a relatively common health condition, affecting nearly (7-15) % of all couples. Clinically, it is a highly heterogeneous pathology with a complex etiology that includes environmental and genetic factors. It has been estimated that nearly 50 % of infertility cases are due to genetic defects[2]. The syndrome androgen insensitivity is a disorder where there is resistance to androgen actions influencing both the morphogenesis and differentiation of androgen responsive body structures, this disorder includes a spectrum of changes ranging from male infertility to completely normal female external genitalia in a chromosomally male individual[3].

The androgen insensitivity syndromes (AIS) comprise a fascinating group of X-linked recessive disorders of sex development (DSD), known collectively as 46 XY DSD, and identification the syndrome as "testicular feminization syndrome" in 1953 by Dr. John Morris[4].

Androgen insensitivity syndrome (AIS) or testicular feminization is a partial or complete inability of cell response to androgen. The cause is enzymatic defect in synthesis of testosterone, resulting sexually immature phenotypically female, with primary amenorrhea. There are three categories of AIS, complete, partial and mild, depending on the degree of external genital masculinization[5].

While a nucleotidic substitution at the level of exon 1 in the position 540 (CAG→TAG), generates the substitution of the glutamic acid with a stop sequence. As a result there is lack of production of the androgen receptor, genetic condition associated with "complete androgen insensibility syndrome"[6].

The Mild type of syndrome represents the opposite end of the AIS continuum. It currently represents an underexplored area full of potential for new discoveries. MAIS has been identified in a small subset of men with male factor infertility characterized by oligospermia or azoospermia, normal serum testosterone levels and elevated LH concentrations. Genetic studies have demonstrated a defect of the androgen receptor gene[7].

Formation of spermatozoa occurs in a sequential manner with mitotic, meiotic, and post-meiotic differentiation phases each of which is controlled by an intricate genetic program. Genes control a variety of physiologic processes, such as hypothalamus-pituitary-gonadal axis, germ cell development, and differentiation[8].

The gene has a length of about 90kb and includes 8 exons. The exons are separated by 7 introns. This gene codifies the AR receptor protein that is composed by 919 amino acids with a molecular weight of 110-112 Kd,the protein is characterized by four functional regions[9].

The exon (1) function is N-terminal transactivation domain (TAD): long region NH2-terminal[10].as shown in Figure(1).

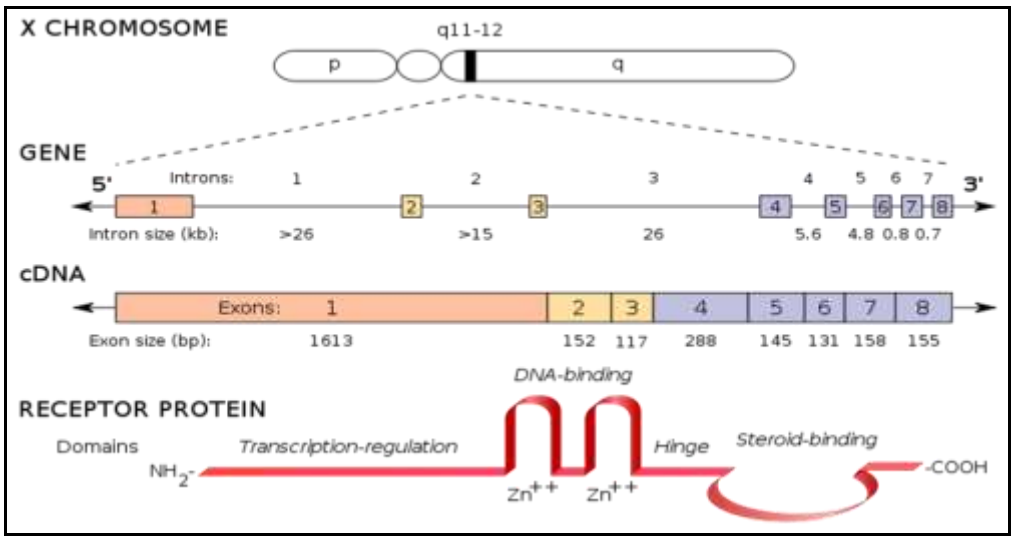

Figure:1 Location and structure of the human androgen receptor. Top, The AR gene is located on the proximal long arm of the X chromosome. Middle, The eight exons are separated by introns of various lengths. Bottom, Illustration of the AR protein, with primary functional domains labeled (not representative of actual 3-D structure).

The AR NH 2-terminal domain harbours the major transcription activation functions and several structural subdomains. Within its 538 amino acids, two independent activation domains have been identified: activation function 1 (AF-1) located between residues 101 and 370 that is essential for transactivation potential of full-length AR and AF-5 (located between residues 360 and 485) that is required for transactivation potential of a constitutively active AR which lacks the LBD[11].

The hormone-dependent interaction of theNH2-terminal domain with the ligand-binding domain can play a role in stabilization of the AR dimer complex and in stabilization of the ligand receptor complex by slowing down the rate of ligand dissociation and consequently decreasing receptor degradation[12].

However, the somatic Sertoli, PTM, Leydig, vascular endothelial and vascular smooth muscle cells of the mature testis express androgen receptor[13], and it is widely accepted that the requirement of testosterone for spermatogenesis is mediated by these cell types.

The AR-mediated androgen actions play key roles in the development and maintenance of male and female phenotype and reproduction function[14].

Given the complicated structure and the important function of each AR domain, it is not surprised that AR alternative splicing would affect the AR cellular signal and result in pathologic conditions. Numerous alternative spliced AR isoforms have been identified in different pathologic conditions including prostate cancer, Kennedy disease, androgen insensitivity syndrome (AIS) and so on[15].

2. Material and Method:

Genomic DNA was isolated from blood cells under aseptic condition according to the protocol described by **Geneaid Biotechnology** Company for wizard genomic DNA purification kit. The sense primer of exon 1 was synthesized as follows: 5' CGG GTT CTC CAG CTT GAT GCG 3'. The antisense primer was 5'GCT CCC ACT TCC TCC AAG GAC AATTAC 3'.

The components of PCR deionized water (9 µl), Green Master mix (12.5 µl), F-Primer (1µl), R-Primer (1µl) and DNA sample (1.5µl) at total volume 25µl. The optimization of amplification was performed under the following conditions**Tables (1).**

Table (1): Optimization of PCR conditions for exon (1) of Androgen receptor gene.

Name of cycle	Temperature °C	Time	No of cycle
Initial danaturation	95	3 min	1 cycle
Denaturation	95	1 min	35 cycle
Annealing	55.3	1 min	35 cycle
Extension	72	1 min	35 cycle
Final extension	72	5 min	1 cycle
Soak	4	-	1 cycle

The solutions were prepared to transferred DNA by dissolved agarose 1 mg per 100 ml of T.B.E, while to transferred PCR products the solution performed by dissolved agarose 1.5 per 100 of T.B.E buffer[16].

Electrophoresis was then run for 10 min at 30v and 70v for 1hr, and when the electrophoresis was completed the gel was placed on a UV transilluminator[17], and the DNA ladder marker (50 - 1000 bp) used for correlation from Promega company USA.

3. Results and Discussion:

The results embedded in table (2) expressed the presence or absence (wild type or deleted) respectively of exon (1) in the PCR test figure (2), for different groups of infertility and control.

The results showed that all patients in oligozoospermia group appeared a positive result for presence of exon (1) in a highly significant differences ($P<0.01$) as compared to the negative result absence of exon (1) as well as in control group.

In the same context, the present study registered highly significant differences (P<0.01) between wild type and deletion of exon (1) in both Oligoasthenozoospermia and Azoospermia groups.

On the other hand, the results showed the highest percentage of exon (1) deleted was in Oligoasthenozoospermia group of infertility as compared with other infertility groups and control.

Table (2): Distribution of Exon (1) in different groups of infertility and control group.

The groups	PCR/exon 1	No.	Percentage (%)	Chi-square
Control	Wild type	30	100.00	14.75 **
	deleted	0	0.00	
Azoospermia	Wild type	34	87.18	13.63 **
	deleted	5	12.82	
OligoZoospermia	Wild type	16	100.00	14.75 **
	deleted	0	0.00	
OligoAsthen-zoospermia	Wild type	27	60.00	8.250 **
	deleted	18	40.00	
Total	Wild type	107	82.30	
	deleted	23	17.69	
** (P<0.01).				

** (P<0.01) means highly significant differences in each group.

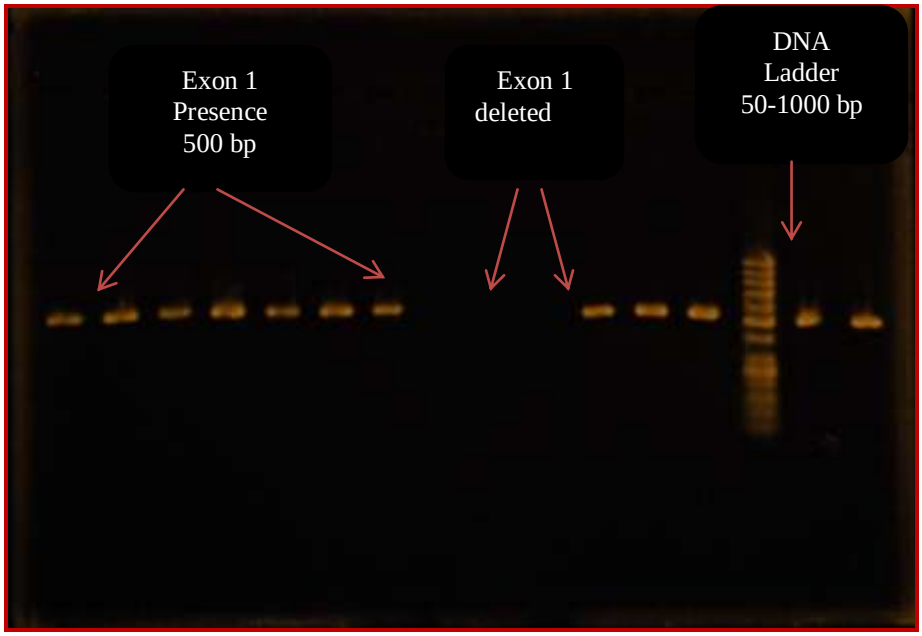

Figure (2):show the presence of exon (1) bands on the left and right of the ladder and some bands lacked.

The results were embedded in table (3) showed the effect of the presence or absence of exon (1) on the hormonal values and semen parameters enrolled in the present study.

Table (3): shown the effect of the presence or absence of exon (1) on the hormonal values and semen parameters.

Parameters	Mean ± SE		T-test
	Wildtype /exon 1	deleted / exon 1	
Testosterone(ng/ml)	4.14 ± 0.17	4.12 ± 0.45	0.816 NS
FSH(mlU/ml)	5.74 ± 0.34	6.62 ± 0.75	1.537 NS
LH(mlU /ml)	3.94 ± 0.16	3.84 ± 0.37	0.757 NS
TSH(mlU/ml)	1.486 ± 0.06	1.523 ± 0.14	0.280 NS
T3(nmol/L)	115.37 ± 3.01	117.29 ± 6.84	13.778 NS
T4(nmol /L)	84.49 ± 1.73	87.73 ± 5.12	8.526 NS
Semen volume (ml)	2.21 ± 0.09	1.89 ± 0.13	0.393 NS
Liquefaction time	37.28 ± 1.02	41.08 ± 2.15	4.502 NS
Sperm count	14.79 ± 3.01	5.56 ± 0.91	11.98 NS
Sperm morphology	31.17 ± 2.53 a	21.28 ± 2.41 b	9.382 **
Sperm motility	38.05 ± 3.69 a	20.44 ± 1.78 b	13.27 **
Progressive motility	18.50 ± 3.12 a	1.11 ± 0.75 b	10.983 **
Sperm A %	3.02 ± 0.80 a	0.00 ± 0.00 b	2.872 **
Sperm B %	15.43 ± 2.51 a	1.388 ± 1.13 b	8.814 **
Sperm C %	19.56 ± 1.82	19.33 ± 1.83	6.895 NS
Sperm D %	62.03 ± 3.78 b	77.89 ± 2.80 a	13.790 **
** (P<0.01), NS: Non-significant.			

**** (P<0.01) means highly significant differences with different letters in Rows.**

(a,b) represents the significant differences between values in parameters.

When compared between the patients group with presence (wildtype) of exon (1) and those with absence (deleted) of exon (1), the results showed no significant differences between the hormonal values and some of semen parameters (semen volume, liquefaction time and sperm count).

In contrast, the results registered highly significant differences (P<0.01) in the other semen parameters (sperm morphology, motility, progressive motility and sperm grades A, B, D), which decreased in patients group lacked (absence) of exon (1), while the grade (C) increased in this group.

The androgen receptor (AR), encoded by AR gene, is a steroidreceptor transcription and regulator factor that mediates the cellular activities of testosterone (T) and dihydrotestosterone (DHT)[18].

The AR-mediated androgen function plays important roles in the development and maintenance of male and female phenotype and reproduction activity[19].

The AIS is represented by a variety of phenotypes ranging from male infertility to completely normal female external genitalia with 46XY for both condition[20].

The alternative splicing of androgen receptor may be a critical pathogenic mechanism in human infertility[21].

Moreover, the exon (1) have important role in the androgens activity within the cells, that the exon 1 encodes N-terminal domain (NTD) [18], and this domain contains the major transactivation activity of the AR, called activation function 1 (AF-1) contains two trans-activating regions, transcriptional activation unit-1 (TAU-1) and 5 (TAU-5), which is indispensable for AR activation and regulates AR gene transcription[22].

In adult males, the function of testosterone on seminiferous tubules is important for the complete maturation of normal sperm, in a process known to be largely mediated by androgen/AR mediated gene changes. However, the downstream mechanisms underlying the functions of AR on Sertoli cells are not yet fully discovered, but SOX9 is needed to regulate several aspects of testicular development and function in mouse models[23].

The age- and stage-specific existence of SOX9 in the testicular cords and seminiferous tubules proposes that SOX9 may play a axillary role in germ cell differentiation[24].

However, the importance of SOX9 has not yet been in human testis, and we do not yet fully understand the physiological relevance of SOX9 in normal and pathological human testes. The studies revealed that SOX9 is highly produce in testiculopathic testes with defected spermatogenesis from Sertoli Cell Only Syndrome (SCOS) patients compared to healthy human testes with normal spermatogenesis, mechanistically, the decreased androgen/AR expression induce the ability of SOX9 to simulate anti mullerian hormone (AMH) production in Sertoli cells[25].

The androgen insensitivity syndrome (AIS), because of an inactivating mutation of the AR, the testes may exhibit some degree of dysgenesis, and are at elevated risk of developing cancer in testicular germ cell[26].

The ablated AR by Endocrine disruption pathways in male sexual differentiation, and transgenesis, both show 30–50% decreasing in Sertoli cell number at around the time of birth and a more severe decreasing (60–75%) in adulthood[27].

Another study in newborn rats has shown that interference with androgen action neonatally, via administration of the AR antagonist flutamide, also decrease Sertoli cell number[28].

These results appear that androgens exert a proliferative action on Sertoli cells during the perinatal period, and the Sertoli cells do not express AR for all or most of this period[29].

Regardless of the mechanism of such influence, recognition the deficiency in androgen production or action in foetal or early postnatal life is the important point, likely to result in a testis with fewer Sertoli cells. As Sertoli cell count per testis determines how many germ cells can be supported through maturation into spermatozoa[30], it can be appreciated idea that effects on sperm counts and/or fertility in an impaired individual are then a possibility.

The leakage of the free radicals makes mitochondria a major intracellular source of reactive oxygen species (ROS). These unique features are probably the cause of faster accumulation of sequence variations in mitochondrial DNA than in nuclear DNA[31.] The PCR amplification of mtDNA has shown a higher incidence of mtDNA deletion in asthenozoospermic patients as compared with unaffected individuals[32].

This excessive ROS production may induce the opening of membrane permeability transition pores and release of free radicals, cytochrome C and other apoptogenic factors that ultimately lead to apoptosis. Although mtDNA mutations have been identified in many studies, their role as a diagnostic marker in male infertility is still under debate. Nonetheless, male infertility due to mtDNA mutation can be successfully treated by ICSI, as mtDNA mutations are not transmitted to the offspring[33].

The results demonstrated the necessity of the exons (1) presence and integrity of the AR function and spermatogenesis process, and the young men were highly sensitive to the alterations in the AR gene. So it is necessary to put the detection presence and integrity of both exons (1), as well as chromosomes aberrations in the infertility prediction and diagnosis for infertility in men.

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