

Gonadotropin and Testosterone hormone's serum levels and partial deletions in the AZFc region in Iranian oligozoospermia infertile males

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ABSTRACT

To investigate the relation of luteinizing hormone (LH), follicle-stimulating hormone (FSH) and Testosterone serum levels with partial deletions in the AZFc region in Iranian oligozoospermia males. **Material and methods:** thirty infertile oligozoospermia and 52 Iranian fertile men included. The hormonal assays were measured by the Radioimmunoassay (RIA). Multiplex polymerase chain reaction (M-PCR) using eight sequence-tagged site (STS) markers were measured on the Yq11 chromosome. **Results:** The mean of FSH and LH levels in all oligozoospermia males were higher than fertile men ($p < 0.001$) and testosterone was lower significantly ($p < 0.001$). Five patients showed partial deletions in AZFc region (four had gr/gr and one had b2/b3 deletions). Six fertile men showed partial deletions (five gr/gr and one b2/b3) with higher level of FSH, LH in their group ($p < 0.05$). **Conclusion:** According to high incidence of partial deletions in the AZFc region among Iranian oligozoospermia males, hormonal assay and molecular screening should be advised before considering for ART treatments.

Keywords: Oligozoospermia; Gonadotropin Hormone; AZFc Partial Deletion

1. INTRODUCTION

Male fertility is the end results of numerous and varied processes which all must act in perfect concert to allow the deposition of healthy sperm in to the female

reproductive tract. If any of these co-dependent steps is faulty, infertility may result.

At least 30% - 50% of couples with primary infertility will have a male related problem as the sole or partial reason for their infertility. Germ cell development is dependent on the balanced endocrine interplay of the hypothalamus, the failure of pituitary to secrete FSH and LH will result in disruption of testicular function leading to infertility [1-3]. In addition, Yq, long arm of Y chromosome, contains at least one gene that is responsible for spermatogenesis [4]. Several genes are located in the azoospermia factor region (AZF) on four major intervals defined as AZFa, AZFb, AZFc and AZFd [5-8]. Partial deletions of the AZFc region are reported as a significant risk factor for oligozoospermia. AZFc, the most frequently known genetic causes of impaired spermatogenesis, is mapped between deletion intervals 6C and 6E on Y chromosome.

Deletion in this region is found in 2% - 10% of azoospermic or severely oligozoospermic men [5,8]. The AZFc region removes 3.5 Mb and contains seven gene families which have an important role in spermatogenesis [9]. The existence of the three subdeletions; namely gr/gr, b1/b3 and b2/b3 have been confirmed by using the new molecular markers [10].

The AZFc region is occupied halfly by the gr/gr type, which spans 1.6 Mb; it is thought to be a risk factor for spermatogenic failure [11]. It is found that the direct homologous recombination causes this kind of the subdeletion which also accounts for another type, b1/b3 deletion (1.6 Mb) [10,11]. An inversion event is responsible for two other types which are belonging to the 2nd class. The gr/rg inversion is followed by a b2/b3 deletion (1.8 Mb), while the b2/b3 inversion is followed by a gr/gr deletion (1.8 Mb) [12] or g1/g3 deletion (2.2 Mb) [11].

The aim of this study is to investigate Testosterone, FSH, LH serum levels and the prevalence of partial deletions in the AZFc region (gr/gr, b1/b2, and b2/b3) in Iranian oligozoospermia males.

2. MATERIAL & METHODS

Thirty infertile oligozoospermia with at least 3 year's duration of infertility referred from Mirza Kouchek Khan hospital and Koasar fertility and impotency center. In all the oligozoospermia cases sperm concentration was (<20 mil/ml) with normal karyotypes. Female partners were examined for their fertility using hormonal evaluation for ovarian function and laparoscopy for the confirmation of tubal patency. Only those couples where the male partner was found to be oligozoospermia and the female was normal selected for the study. Fifty two normal fertile men with normal karyotypes and sperm concentration (<20 million/ml) were selected as control group. The hormonal assays were measured by the Radioimmunoassay (RIA), and for the presence of eight sequence-tagged site (STS) markers on the Yq11 chromosome, Multiplex polymerase chain reaction (M-PCR) was used. An informed written consent was obtained from all subjects for molecular screening and hormonal Assay.

2.1. Specimen Collection

10 ml of peripheral blood was collected in a sterile tube containing ethylene diamine tetra acetate (EDTA) as an anticoagulant and divided into two parts:

- 1) 7 ml was centrifuged (2500 g rpm) for 5 minutes and serum was stored at -20°C for hormonal assay.
- 2) 3 ml was used for DNA extraction.

2.2 Assay Hormones

Serum levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH) and testosterone were collected early in the morning and evaluated at the Mirza kouch khan hospital pathology reference services.

In all subjects, serum levels of LH were measured by radioimmunoassay kit (Amersham Corp., Arlington Heights, Illinois, USA). Reference ranges were 4 to 23 mIU/ml. Similarly, serum levels of FSH were determined by radioimmunoassay kit. Reference ranges were 10 to 76 mIU/ml. and for serum levels of testosterone were determined by radioimmunoassay kit (Amersham Corp., Arlington Heights, Illinois, USA) with reference ranges were 0.2 to 11 ng/ml.

2.3. Molecular Screening

Genomic DNA was extracted from peripheral blood

using Tadbir Azma DNA extraction kit (Tadbir Azma, Tehran, Iran). First of all, we investigated classical AZF microdeletions; consequently, the polymerase chain reaction was used to detect 8 different sequence tag sites (STS) corresponding to different AZF loci according to the European Academy of Andrology (EAA), the European Molecular Genetics Quality Network (EMQN) (13). These included sY84 and sY86 (AZFa); sY127 and sY134 (AZFb); sY254 and sY255 (AZFc); and sY145 and sY153 (AZFd). These STSs were analyzed using three different M-PCRs:

Multiplex 1: sY86, sY127, sY254, and ZFY;

Multiplex 2: sY84, sY134, sY255, and SRY;

Multiplex 3: sY145, SY154, and SRY.

Two primers (ZFY, SRY) were used as internal controls for M-PCR. Primers sequences with additional details are provided in a previous study [14]. The reaction was comprised of 50 - 100 ng of genomic DNA in 25 ul PCR reactions, 10 mM Tris HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 2 mmol dNTPs, 10 pmol of each primers and 1 unit of Taq DNA polymerase. Initial denaturation step at 94°C for 5min, followed by 28 cycles of 94°C for 30 sec, 56°C for 30 sec and 72°C for 45 sec ending with a final extension for 5 min at 72°C .

The PCR products were separated on 2% agarose gels stained with ethidium bromide, or 10% acrylamide gel prepared in 0.5X TBE. In all reactions, DNA from a normal woman and a normal man were included as controls.

The samples without classical AZF deletions were investigated in order to find gr/gr, b1/b2, and b2/b3 polymorphisms. The presence or absence of these subdeletions was analyzed using M-PCR. Two STS markers were detected for the study and were tested in one M-PCR: SRY, SY1191, and SY1291. The sequence of the primers is shown in **Table 1**. Reaction condition and PCR program was similar to the program used for detection of classical AZF. The PCR products were separated on 2% agarose gel. The absence of amplification of sY1291 represented a gr/gr deletion and the absence of sY1191 product showed b2/b3 deletion. Deletion of the two markers was defined as b1/b3 deletion.

2.4. Statistical Analysis

Independent Student's t-Test was used to compare the numerical values of blood plasma hormonal assay in both groups. All procedures were carried out using SPSS for Windows V11.5. The type 1 error was set at 0.05 for testing the hypothesis.

3. RESULTS

The FSH and LH levels in oligozoospermic group

men were significantly increased ($p < 0.001$). The mean value of testosterone levels significantly decreased in oligozoospermic men in compare with fertile men ($p < 0.001$) (Table 2).

Totally, only 1 out of 30 oligozoospermic men (3.3%) with spermatogenesis impairment was found to have classical AZFb deletion. The gr/gr, b2/b3 and b1/b3 subdeletions in all subjects were determined using M-PCR.

After having eliminated 1 classical AZFb deletions from 30 oligozoospermic men, subdeletions in the AZFc region; was found in 4cases (13.8%) gr/gr subdeletions, 1(3.4%) b2/b3 subdeletions and 0(0%) b1/b3 subdeletions.

In contrast, screening of 52 control fertile men revealed 5(9.6%) gr/gr subdeletions, 1(1.9%) b2/b3 subdeletions and 0(0%) b1/b3 subdeletions. There was no significant difference in the frequency of the gr/gr and b1/b3 subdeletions between the patients and controls ($p > 0.05$).

The mean value for FSH, LH and testosterone concentration in normal fertile men was 7.48 mIU/ml, 6.73 mIU/ml and 3.93 ng/ml respectively (Table 2). In 4 oligozoospermia infertile men with gr/gr subdeletion; FSH, LH was higher and clearly subnormal testosterone is shown (Table 3).

4. DISCUSSION

Hormonal assay of the FSH, LH and testosterone is useful in the management of the male infertility, and for initiation of spermatogenesis and maturation of spermatozoa [3,15]. In the oligozoospermic men higher concentration of FSH is considered to be a reliable indicator of germinal epithelial damage or primary testicular failure [7,8]. Moreover, different studies have reported that serum FSH and LH were significantly above the mean value in infertile oligozoospermic men with Y-chromosome microdeletion [6,16].

In the present study, the same results were obtained and FSH, LH in oligozoospermia patients were significantly higher than fertile group and testosterone values was lower ($p < 0.001$), this indicates primary testicular failure in our oligozoospermia men. Five oligozoospermia patients showed partial deletions in AZFc region (4 had gr/gr and one had b2/b3 deletions).

Combination of gr/gr subdeletion and sub normal testosterone serum level was not explained by any other investigators [17,20]. In opposite of some previous studies [17,18,20], these values were significantly different in infertile oligozoospermia males with AZFc subdeletions. Different results obtained from the present study

Table 1. STS markers and primer sequences used for screening gr/gr, b1/b2, and b2/b3 polymorphisms.

STS	AZF	Production Size(bp)	sequence
sY1191	AZFc	385	5'-CCAGACGTTCTACCCCTTCG-3 5'-GAGCCGAGATCCAGTTACCA-3'
sY1291	AZFc	527	5'-TAAAAGGCAGAACTGCCAGG-3' 5'-GGGAGAAAAGTCTGCAACG-3'

Table 2. Serum FSH, LH and testosterone levels in fertile and oligozoospermic men.

Group	No. subjects	Hormonal levels mean(SD)		
		FSH (miu/ml)	LH (miu/ml)	Testosterone (ng/ml)
Fertile men	52	7.48 ± 4.21 Range (2.50 - 18)	6.73 ± 3.73 Range (2.10 - 18.30)	3.93 ± 1.09 Range (2.20 - 8.25)
Infertile men (Oligozoospermia)	30	20.28 ± 11.59* Range (4.50 - 40)	14.67 ± 7.29* Range (3.80 - 35)	3.11 ± 1.67* Range (0.80 - 8.50)

* $p < 0.001$.

Table 3. Clinical features of 4 oligozoospermia men with gr/gr subdeletion.

Patient No	Sub deletion	FSH mIU/ml	LH mIU/ml	Testosterone ng/ml	Sperm count mil/ml
7	gr/gr	36	15	2.60	1 × 10 ⁶
8	gr/gr	37	20	2.60	1 × 10 ⁶
22	gr/gr	40	17	1.50	5 × 10 ⁶
26	gr/gr	37	22	2.50	3 × 10 ⁶

may be due to limitation in sampling or oligozoospermia classification.

The FSH producing by pituitary is affected by the Inhibin B feed-back control [17], which is produced by the sertoli cells. In the patients with the germinal cells function deficiency, the inhibin rate will be reduced while the FSH rate will be increased. Indeed, this is a possible interaction between the germinal and sertoli cells. The serum FSH rate is related to the epithelium function of the germinal cells [18]. Additionally, It is commonly approved that complete deletion of Y chromosome is a determining factor of sever spermatogenesis disorder. And the relationship between partial deletions of AZFc region and male sterility is to be discussed [6,19].

Furthermore many studies have been carried out for detecting the relationship between subdeletions and sterility in recent years [13,14,18,20]; however the results in different populations were not identical. In the present survey no significant differences were detected for the frequencies of gr/gr and b2/b3 deletions in the studied groups ($p > 0.05$).

According to the surveys carried out on a population in East Asia the frequency of gr/gr deletion in terms of prevalence percentage were estimated respectively as 10.3% and 10.1% [12,21], that is comparable with our result. The only deletion which is created through gr/gr recombination is the significant cause of spermatogenesis failure [14].

Men with AZFc subdeletion are somatically healthy with most likely have useable sperm, stable sperm production over time and a good chance to experience biological paternity. However, their sons would also be AZFc subdeletion carrier [14,16,19,20]. In our study, b2/b3 frequency equated to 3.4% showing no significant difference in comparison to the frequency of these subdeletions in fertile population. In all the previous studies, no significant relationship was detected between the prevalence of this deletion and sterility which can be due to the racial differences among populations [19,21]. Furthermore the present study may be in conflict with similar studies or other surveys [14,19]. The role of b2/b3 deletions in male sterility is still unknown because of its low frequency. It seems that the role of DAZ3/4 deletion in male sterility is limited to the b2/b3 subdeletion [14,22]. Therefore; more studies should be done for detecting the role of gr/gr deletion and b2/b3 subdeletion in the context of Y haplogroups. However, there are different factors which influence the results of the studies. Observed differences in the deletions of AZFc region in various studies can be due to diverse demographic composition, differences in criteria for selection of patients, environmental impacts and also the categorization method applied for the definition of oligozoospermia due to dif-

ferent classification depend on the sperm count and characteristics [22]. Vogt considered the oligozoospermic criteria less than 2 mil/ml sperm in his study [7]. In Other investigations oligozoospermic criteria was considered less than one mil/ml, which obviously can highly influence the results of the studies [23,24]. In our study less than 20 mil/ml was considered as oligozoospermia, which may cause significant increase in the amount of gonadotropin hormones and decreased testosterone serum level in compare to normal fertile group. Our study revealed that FSH, LH, and testosterone evaluation is useful in the management of male infertility, but the couples with a high risk transferring of a main genetic defect recommended performing molecular screening of AZF partial or subdeletion before any decision for generation.

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