STUDY ON CLINICAL AND GENETIC CHARACTERISTICS OF MALE PATIENTS WITH NON - OBSTRUCTIVE AZOOSPERMIA

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We examined 501 patients with non - obstructive azoospermia to evaluate clinical, subclinical, and genetic characteristics. The results show that the average age of patients in the study was 29.8 ± 5.5 years. Primary infertility accounts for the majority, with a rate of 90.3%. There was 38.6% of patients had a history of mumps orchitis. The average levels of FSH, LH, testosterone were 31.6 ± 16.5 mIU/mL, 15.5 ± 10 mIU/mL and 12.8 ± 7.13 nmol/L, respectively. The prevalence of chromosomal abnormalities was 30.7%. Of these, the sex chromosome aneuploidy with 47,XXY karyotype (Klinefelter syndrome) accounted for 27.3%. The incidence of AZF microdeletion was 13.8%. Of these, AZFc deletion was the most common at the rate of 42.1%, AZFa deletion, which accounted for 2.6%, were the least prevalent, and the frequency of AZFd deletion was 5.3%. However, there was no solitary AZFb deletion, which combined with other AZF deletions with 34.2%. Our research shows that mumps orchitis and chromosomal abnormalities are the leading causes of azoospermia. Screening for genetic abnormalities plays an important role in infertile patients with non - obstructive azoospermia.

Key word: Azoospermia, Non - obstructive azoospermia, Chromosome, AZF

I. INTRODUCTION

Recently, infertility is gradually becoming a burden to society, as well as the concern and the anxiety of infertility couples. According to previous studies, the infertility rate accounts for about 15% of couples.[1] In cases of male infertility, men with azoospermia have the most severe impact on reproductive function, making it impossible for men to spontaneously father a child and have to rely on assisted reproductive technology.[2]

Among azoospermic men, patients with non - obstructive azoospermia (NOA) have very serious damage to the spermatogenesis. Testicular sperm extraction (TESE, micro - TESE) associated with IVF / ICSI is the only option for the treatment of infertility in infertile patients with NOA. However, TESE have not been unable to find sperm in all patients. Multiple markers are believed to have a predictive value on the success of obtaining sperm from the testes were studied, such as age, body mass index (BMI), medical history, testicular size, genital malformations, hormonal parameters, and genetic abnormalities.[3] In clinical practice to reduce the economic burden of treatments and to avoid unnecessary interventions, it is essential to understand the clinical and subclinical features of infertile patients with NOA which provide the clinicians information to have appropriate treatment directions and prognosis for each case.

In Vietnam, up to now, there have been only a few small studies on male infertility with azoospermia.[4,5] These studies focused mainly on the results of treatment for infertility

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patients. The number of studies on the clinical and subclinical characteristics of NOA patients is limited. Therefore, we proceed to the topic: "Study on clinical and genetic characteristics of male patients with non - obstructive azoospermia" to the following objectives:

1. Description of the clinical and subclinical characteristics of infertile patients with non - obstructive azoospermia.

2. Identification of genetic abnormalities associated with non - obstructive azoospermia.

II. METHODS

1. Subjects

The study was conducted on infertile male patients without spermatozoa examined at Hanoi Medical University Hospital from April 2013 to October 2019.

Inclusion criteria:

- Patients of reproductive age from 18 - 55 years old.

- The patient performed 2 semen samples but there were no sperm in the semen.

- Patient was diagnosed as non - obstructive azoospermia according to Huang I.S's criteria[6] with FSH > 9.2 mIU/mL, and on ultrasound the mean bilateral testicle size was less than 15 mL.

- Patients were fully evaluated for clinical, subclinical and genetic characteristics.

2. Method

Study design: Cross - sectional descriptive study.

Study sample: Convenient sampling.

Study procedure:

All subjects undergone a thorough process to determine the cause of azoospermia, including interviewing medical history, marital status, duration of infertility and the examination of external genitalia.

Semen analyses were conducted at the Department of medical biology and Genetics – Hanoi Medical University. There were a 3 - 5 days of abstinence between the collection of the two semen samples.

Human karyotyping and AZF microdeletion analysis were also conducted at the Department of medical biology and Genetics – Hanoi Medical University. The karyotype was determined by leukocytes cultured from peripheral whole blood and scanned under electron microscope with Metafer scanning system. Microdeletion of AZF was determined by multiplex PCR technique using the AZPA_D kit.

3. Ethics approval

The study was approved by the Ethical Review Committee of Hanoi Medical University. Information of the participants is confidential.

4. Statistical analysis

Data processing using R software. Using descriptive statistics of quantity, percentage, mean, standard deviation, Min, Max and p value of < 0.05 was considered to be statistically significant.

III. RESULTS

1. Clinical characteristics of the research subjects

The mean age of the study subjects was 29.8 ± 5.5 years. The proportion of patients with a history of mumps - orchitis accounted for 38.6%. The majority of men was primary infertility.

Table 1. General characteristics of the research subjects

Characteristics	*N*	*%*	*Mean ± SD*	*Median*	*Min - Max*
Age (year)	501		29.8 ± 5.5	29.0	15.0 - 51.0

Characteristics	*N*	*%*	*Mean ± SD*	*Median*	*Min - Max*
Height (cm)	501		167.7 ± 6.02	168.0	139.0 - 187.0
Weight (kg)	501		62.4 ± 9.22	62.0	41.0 - 100
BMI (kg/m2)	501		22.2 ± 2.92	22.0	15.7 - 33.3
< 18.5	54	11.2%			
18.5 - 23	257	53.3%			
> 23	171	35.5%			
Smoking	444				
Yes	99	22.3%			
No	345	77.7%			
History mumps orchitis	306				
Yes	118	38.6%			
No	188	61.4%			
Infertility classification	371				
Primary	335	90.3%			
Secondary	36	9.7%			

2. Subclinical characteristics of the research subjects

Table 2. Subclinical characteristics of the research subjects

Characteristics	**n**	**%**	**Mean ± SD**	**Median**	**Min - Max**
LH (mIU/mL)	501		15.5 ± 10	12.2	1.80 - 63.2
< = 7.6	105	21.0%			
> 7.6	396	79.0%			
Testosterone (nmol/L)	501		12.8 ± 7.13	11.6	0.43 - 48.3
< 12	265	52.9%			
> = 12	236	47.1%			
Right testicular volume (mL)	501		5.56 ± 3.51	5.44	0.37 - 14.9
< 12	479	95.6%			
≥12	22	4.4%			
Left testicular volume (mL)	501		5.52 ± 3.34	5.44	0.05 - 14.8
< 12	482	96.2%			
≥12	19	3.8%			

Characteristics	n	%	Mean ± SD	Median	Min - Max
Semen analysis					
pH	487		7.49 ± 0.44	7.50	5.00 - 9.00
< 7.2	46	9.4%			
> = 7.2	441	90.6%			
Volume (mL)	493		2.47 ± 1.4	2.50	0.20 - 9.80
< 1.5	119	24.1%	31.6	29.2	9.23 - 131.2
> = 1.5	374	75.9%	5.56	5.44	0.37 - 20.1

The study group's serum FSH and LH concentrations were higher than normal values, while the average serum testosterone concentrations were within the normal range. The research group's mean bilateral testicular volume was smaller than the normal limit. Average pH and semen volume of the study subjects were mostly within the normal range.

3. Genetic characteristics of the research subjects

Table 3. Genetic characteristics of the research subjects

Characteristics	N	%
Sex chromosome aneuploidy	80/286	28.0%
XO - XY mosaic (XO/XY)	1/286	0.3%
Klinefelter syndrome (47,XXY)	78/286	27.3%
48,XXXY syndrome	1/286	0,3%
Structural chromosome abnormalities	6/286	2.1%
Chromosomal polymorphism	1/286	0.3%
46,XX karyotype	1/286	0.3%
46,XY karyotype	198/286	69.3%
Total	286	100%

The results of chromosome mapping observations of 286 patients showed that the rate of chromosome abnormalities accounted for 30.7%, most of them were numerical mutations accounting for 28%, of which Klinefelter syndrome accounted for the proportion of the highest 27.3%.

Table 4. Characteristics of AZF microdeletion of Y chromosome

Characteristics	N	%(N = 276)	%(n = 38)
AZF a	1/276	0.4%	2.6%
AZF c	16/276	5.8%	42.1%
AZF d	2/276	0.7%	5.3%
AZF c + d	6/276	2.2%	15.8%

Characteristics	N	%(N = 276)	%(n = 38)
AZF b + c	2/276	0.7%	5.3%
AZF b + c + d	6/276	2.2%	15.8%
AZF a +b + c	3/276	1.1%	7.8%
AZF a +b + c +d	2/276	0.7%	5.3%
Total	38/276	13.8%	100%

Of the 276 patients had done an AZF mutation showed that the AZF microdeletion accounts for 13.8%.

IV. DISCUSSION

In clinical practice, it is very important to accurately diagnose the cause of azoospermia. It helps doctors to prognose and comes up with suitable treatments for infertile men. However, it is not easy to distinguish between azoospermia causes. To diagnose the cause of the absence of sperm, testicular biopsy plays a decisive role, but this method is invasive and has many risks of complications. In order to avoid unnecessary interventions, we use the reference value in the Huang I.S study as the criteria for selecting NOA patients based on FSH concentration and testicular size for inclusion in the study. In the study of Huang I.S using a combination of FSH value > 9.2 mIU / mL and testicle size < 15 mL, predictive value of NOA is 99.2%.[6]

Exploiting the medical history, we found that up to 38.6% of the study subjects had a history of orchitis due to mumps. Mumps virus has a high affinity for testicular parenchyma, leading to parenchymal edema with spermatic tubular congestion and, consequently, necrosis accompanied by seminiferous tubules hyalinization and testicular atrophy. Previous reports have shown that the complication rate of orchitis occurs in 15 - 40% of men after puberty who are infected with the mumps virus.[7] Up to 30 - 50% of patients had complications and 13% testicular atrophy patients with infertility after complications from orchitis due to mumps. There is a need to raise public awareness about measures to prevent mumps, as well as to have active treatment for patients with mumps orchitis to prevent complications that can affect to reproductive function.

In the regulation of spermatogenesis and testosterone, there is a close relationship in the hypothalamic - pituitary - testicular axis. Therefore, FSH, LH and testosterone are considered as indicators to evaluate the operational function of the testicles. In our study, the average testosterone level in the majority of the study subjects was within the normal range, while the FSH and LH concentration in the majority of the subjects were very high. Goluza's study also showed that testosterone concentration in most patients with NOA was within the normal range and the concentration of FSH and LH increased in the serum.[8] The author believed that in most patients with NOA, there was damages to sperm germ cells and impaired spermatogenesis function. Meanwhile Leydig cells were only partially damaged and had the functional compensation of the remaining normal Leydig cells under the stimulation of high levels of LH in the blood to ensures adequate production of testosterone.

Male infertility is caused by a variety of

pathologies, and studies have shown that cytogenetics and molecular genetic disorders such as an abnormality of chromosomes number or mutations in the Y chromosome are the main genetic causes of male infertility, especially in NOA patients. Investigation on genetic characteristics of our study subjects showed that the rate of chromosomal abnormalities accounted for a high rate up to 30.7%, of which sex chromosome aneuploidy with 47,XXY karyotype in the majority of patients (27.3%). According to previous studies, the rate of genetic abnormalities in infertile azoospermic patients ranges from 20 - 30%, the 47,XXY karyotype accounted for 10 - 20%.[9,10] According to a research by Nguyen Duc Nhu et al (2009), the rate of chromosomal abnormalities in 354 men with azoospermic infertility accounted for 16.7% with 11% of patients had 47,XXY karyotype.[11] Compared with the aforementioned studies, the prevalence of genetic abnormalities and infertile patients with Klinefelter syndrome in our study were higher due to our selection criteria focusing on NOA patients. In general, in all studies, the rate of chromosomal abnormalities, numerical mutations with sex chromosome aneuploidy with 47,XXY karyotype were most prevalent.

In addition to chromosomal abnormalities, molecular genetic disorders including microdeletion of the Y chromosome is the second recognized genetic cause of infertility in men. Previous studies have shown that the incidence of AZF microdeletion of Y chromosomes in infertile men ranges from 5 to 15%,[11] this rate in our study was 13.8%. Deletion between different AZF regions often appear at different rates, Lee J.Y's study showed that the AZFc deletion had the highest percentage for 60%, followed by AZFb which was 16%, 5% for AZFa, and 14% for concomitant microdeletions.[9] In the study of Luong Thi Lan Anh on 30 infertile patients with azoospermia, the rate of AZFc deletion was 46.7%, AZFb deletion was 16.6%, AZFa deletion was not detected, and the AZFd deletion was 6.7%.[4] Compared with our study, it was consistent that the rate of AZFc deletion also had the highest rate with 42.1% and the lowest rate was AZFa with 2.6%. AZFd deletion accounted for 5.3%. We did not detect any cases with solitary AZFb deletion, only in the combination with other fractional mutations with the rate of 34.2%. The differences between studies may be related to different races, population groups, and study subjects. Thus, along with the chromosome test, detecting microdeletion mutations on the Y chromosome deletions has clinical significance in diagnosing the cause as well as predicting the ability to find sperm in NOA patients, helping patients reduce time and treatment cost, as well as avoid unnecessary interventions.

V. CONCLUSSION

In male infertile patients with non - obstructive azoospermia, a history of mumps - orchitis was a relatively high 38.6%, along with genetic abnormalities considered the main causes leading to non - obstructive azoospermia.

The incidence of chromosomal abnormalities, especially the abnormalities of chromosome number with 47,XXY karyotype, and the incidence of AZF deletion was quite high, suggesting the important role of screening testing for genetic abnormalities in infertile patients with non - obstructive azoospermia.

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