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Original Article

Cytogenetic analysis in primary male infertility with oligospermia or nonobstructive azoospermia: Correlation with clinical and endocrine profile

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Abstract

Objectives: Chromosomal anomalies are common in infertile males with oligospermia or non-obstructive azoospermia. The prevalence of chromosomal anomalies in subjects with male infertility and its correlation with clinical and endocrine profile was investigated. *Methods:* Consecutive 91 male subjects (mean age 23.5±5.3 years) with primary infertility were enrolled from a referral genetic center in a developing country. These patients had moderate or severe oligospermia (n=46) or non-obstructive azoospermia (n=45). Clinical (androgenization, testicular volume) and endocrine evaluation (serum FSH, testosterone and prolactin) and prometaphase and metaphase chromosome analyses by phytohemagglutin (PHA) stimulated lymphocyte culture were carried out. *Results:* Ten (10.9%) subjects had chromosomal abnormality. The highest frequency of abnormal karyotypes (15.5%) was found among patients with nonobstructive azoospermia. The most frequent anomaly was 47, XXY chromosomal constitution, found in 4 (4.4%) patients with non-obstructive azoospermia. Majority of the chromosomal aberrations were sex chromosomal type. Autosomal aberration and structural aberration was seen in one patient each. All patients with numerical chromosomal anomalies had nonobstructive azoospermia. *Conclusion:* We observed high prevalence of chromosomal abnormalities in infertile males with moderate or severe oligospermia or nonobstructive azoospermia. We recommend chromosomal analysis should be performed in male infertility from diagnostic, prognostic and therapeutic viewpoint.

Key words: chromosomal analysis, male infertility, karyotype, azoospermia, oligospermia

Introduction

Infertility affects about 15% of all the couples attempting pregnancy with male factor identified in approximately half the cases [1]. Suboptimal male fertility can be caused by various genetic factors affecting male gamete formation or function [2,3]. The reduction in the number of sperm or function may be caused by either a chromosomal or a single gene disorder [2,3]. Higher frequencies of chromosomal abnormalities ranging from 5% to 27% are found in infertile males than in general male population [4,8]. Various chromosomal abnormalities reported are numerical or structural abnormalities of sex chromosomes, Robertsonian translocation, paracentric inversions of autosomes and marker chromosomes [9]. With decreasing sperm counts, there is a progressive increase in the frequency of chromosomal abnormalities which are more common with severe oligospermia and nonobstructive azoospermia than moderate or mild

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oligospermia[10]. The most common karyotypic abnormality in men with oligospermia or azoospermia is Klinefelter syndrome (47, XXY, mosaic). These subjects can be identified clinically by characteristic body proportions, gynecomastia, firm and small testis and associated endocrine and nonendocrine disease [11].

We performed chromosomal analysis in case of male infertility having nonobstructive azoospermia and moderate to severe oligospermia. The karyotype findings were correlated with physical, anthropometric and endocrine profile.

Material and Methods

Consecutive males with primary infertility (n=91) and having nonobstructive azoospermia (n=45) or moderate to severe oligospermia (sperm counts 5-10 millions/ml defined as moderate oligospermia and counts <5 millions/ml defined as severe oligospermia; n=46) were evaluated as a part of routine medical care. Subjects with systemic disorders, liver or kidney disease, or those on androgen or gonadotropins in the last six months were excluded. These patients were subjected to cytogenetic analysis.

All the subjects underwent physical examination with emphasis on assessment of skeletal proportions, androgenization, testicular volume, gynecomastia and varicocele. Semen collection analysis was carried out carried out twice at an interval of two weeks and after an abstinence period of 2-8 days. Semen analysis was carried out as per the criteria set by WHO [12].

Pooled blood samples were collected at an interval of 20 minutes, and serum was separated and stored at -20 C till the time of hormonal assays. Estimation for serum LH, FSH and total testosterone were carried out by chemiluminence method.

Chromosomal analysis was performed from peripheral blood culture by phytohemagglutin stimulated lymphocyte culture as a standard method [13]. G.T.G. banding was performed. Q banding was combined in some cases whenever required. Detailed analysis in 20 metaphases was done, while 100 metaphases were evaluated in each patient for numerical abnormality.

Results

Ten (10.9%) subjects had chromosomal abnormality. In azoospermia group, seven subjects (15.7%) had chromosomal abnormalities. All these subjects had numerical chromosomal abnormalities; four showing 47, XXY karyotype while 3 had 46, XY/47, XXY mosaic karyotype. Oligospermia group had 3 subjects (6.6%) with chromosomal abnormalities; two subjects had 46, XY/47, XXY mosaic karyotype while one had structural (translocation) anomaly 45, XY, t (13:14). A list of chromosomal anomalies in various groups is given in table 1 and 2.

Table 1. Chromosomal anomalies in infertile males with azoospermia and oligospermia.

Group	No. of subject	Numerical sex schromosome anomaly n(%)	Structural sex chromosome anomaly n(%)	Autosomal anomaly n(%)	Normal karyotype
Azoospermia	45	7 (15.5)	0 (0)	0 (0)	38
Oligospermia	46	2 (4.3)	0 (0)	1 (2.1)	43
Total	91	9 (9.9)	0 (0)	1 (1)	81

Table 2.Chromosomal anomalies in azoospermia and oligospermia.

Group	No. of cases	Chromosomal anomaly
Azoospermia	4/45	47, XXY
	3/45	46, XY/47, XXY
Oligospermia	2/46	46,XY/47,XXY
	1/46	45, XYt (13:14)
Total	10	

Testicular volume in all subjects with 47, XXY, karyotype was less than 3 ml, serum FSH levels ranged from 17-110 IU/L and serum LH levels ranged from 2.1-125 IU/L. The testicular volume in patients with mosaic karyotype in either group was 2.5-13 ml and serum FSH levels were ranging from 7-92 IU/ml. LH levels ranged from 1.5-117 IU/L. Rest of the cases with normal karyotype had testicular volume ranging from 3-15 ml and FSH from 1.4 – 70 IU/L. Serum LH levels ranged from 3.2-125 IU/L. (table 3).

Table 3. Physical and endocrine profile with normal and abnormal karyotypes.

Karyotype	Testicular volume (ml)	S.FSH (IU/L)	S.LH (IU/L)
Azoospermia/oligospermia with normal karyotype	3-15	1.4-70	3.2-125
47,XXY, karyotype	<3ml	17-114	2.1-125
47,XXY/46, XY	<3-13ml	7-92	1.5-117

Discussion

Nonobstructive azoospermia and oligospermia are the common causes of male infertility. Various environmental and genetic factors have been implicated for this. Chromosomal anomalies are an important genetic factor responsible for male infertility. Various cytogenetic studies have reported variable frequency (5-27%) of major chromosomal anomalies in male infertility, depending on the criteria of patient selection[4-8]. We evaluated the patients of moderate to severe oligospermia or nonobstructive azoospermia with or without any clinical findings. In our study, 10.9% of the patients had chromosomal anomalies. The major chromosomal anomalies were numerical sex chromosomal anomaly (10/11) while only one patient had autosomal translocation. The major chromosomal anomalies were present in nonobstructive azoospermia group (15.5%) and our results are in agreement with the previous studies [4-8]. In oligospermia group the frequency of chromosomal anomaly was 6.6% in our study.

All the patients with 47, XXY, had eunuchoidal body proportions, gynecomastia, testicular volume <3 ml and FSH levels from 17-114 IU/ml. Among the patients with mosaic 46, XY/47, XXY karyotype, two patients had testicular volume < 3ml, while the rest of the three patients had testicular volume ranging from 4-13 ml. FSH levels in mosaic karyotype group was from 7-92 IU/L. This observation suggests that in mosaic karyotype group the testicular volume and FSH levels may be within normal limits. It is noteworthy that many subjects with Klinefelter syndrome present to the clinician primarily with primary infertility with nonobstructive azoospermia or severe oligospermia. It is essential to identify these subjects with sex chromosomes aberrations as they require in addition to the genetic counseling and treatment of infertility, androgen replacement therapy, identification of associated health problems viz. lower verbal I.Q. neurocognitive difficulties, thyroid dysfunction, diabetes mellitus, predisposition to breast cancer and abnormalities of urinary tract [11].

Earlier studies have reported Robertsonian translocations, paracentric inversion of autosomes, reciprocal translocations, markers chromosomes [9,10] and chromosomal variants in infertile males with oligospermia and nonobstructive azoospermia [5]. The contribution of Y-chromosomal variants and marker chromosomes to alter the carrier's fertility is still debated and requires further studies.

We did not include mild oligospermia and obstructive azoospermia for cytogenetic evaluation as the possibility of abnormal karyotype is 0.5% and 0.8% respectively[10]. Chromosomal analysis is not cost effective in this group of subjects in view of low prevalence of aberrations.

Karyotyping of sub fertile males is important not only from the diagnostic viewpoint but also even more importantly to gain better understanding of the gametogenic impairment associated with chromosomal

anomalies. It is suggested by meiotic studies that spermatogenic breakdown is often related to alterations in the process of chromosomal synapsis. Any condition that can interfere with XY, bivalent formation and X-chromosome inactivation is critical to meiotic process. Furthermore asynapsed region may represent a signal for meiotic checkpoint that eliminates spermatocytes and synaptic errors.

We conclude that chromosomal analysis is an important investigation in male subjects with primary infertility with moderate to severe oligospermia or non-obstructive azoospermia. Many of the subjects with Klinefelter syndrome may have phenotypic feature and can be identified by simple clinical examination. However, in some subjects, correlation may not be present. Subjects with numerical chromosome abnormality may be offered appropriate genetic counseling and assisted reproductive techniques.

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