SHORT REPORT

Evaluation of chromosomal abnormalities and Y chromosome microdeletion in infertile males of 10 families

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Abstract

This study was designed to investigate the hormonal, seminal changes and chromosomal aberrations in cases of male infertility. A total of ten infertile families from Khyber Pakhtunkhwa of Pakistan were included in the study. The families were clinically evaluated by standard criteria; diagnosis of azoospermic and oligospermic males was confirmed. Seminal, hormonal, ultrasonographic and histopathological examinations were carried out for all the affected participants of the study. Karyotyping was performed on peripheral blood lymphocytes according to standard methods. Hormones were altered in six families. Ultrasonographic abnormal finding was observed in six families. Karyotyping analysis revealed numerical aberration in family G (0X) and family I (XXY). The remaining families had no structural or numerical aberration. Y chromosome microdeletion analysis revealed AZFc deletion in both the affected participants of the family C. The remaining families were found normal for microdeletion. The occurrence of chromosomal anomalies and Y chromosome microdeletions among infertile males strongly suggests the need to include these two tests in routine investigations of male infertility cases

Introduction

The failure to achieve pregnancy after one year of unprotective sexual intercourse is infertility. Infertility may be due to defect in the male or female partner. Azoospermia is the absence of spermatozoa in at least two semen samples including centrifuged sediment.[1,2] About 10-15% couples suffer from infertility worldwide.[3,4] Approximately 50% infertility issues are related to males, which accounts to about 1% of the whole male population[3] and 15% in male infertile population.[5]

Azoospermia is mainly classified into three main categories; pretesticular, testicular and hypogonadism. Pretesticular type in which mainly pituitary and hypothalamus is involved. This class of abnormalities may be acquired, congenital (Kallmann syndrome) or secondary in nature.

In testicular azoospermia, spermatogenic failure occurs while pituitary and hypothalamus work normally. It may be congenital such as cryptochidism, spermatogenic arrest, Y chromosome microdeletion and sertoli cell only syndrome. It may be acquired due to torsion, trauma, testicular tumour, infection, surgery, medication, irradiation or varicocele.[6]The pretesticular and testicular types of azoospermia with patent reproductive ducts are present and patent so considered as a subclass of non-obstructive azoospermia (NOA).

There are two causes of hypogonadism; primary testicular failure which is also called hypergonadotropic hypogonadism and secondary testicular failure which is also called hypogonadotropic hypogonadism.[6,7] Elevated FSH and LH and low testosterone indicate spermatogenesis failure.[5] According to Nudell and Turek if vas deferens was palpable and FSH was elevated so it indicates NOA. It has been reported that 50% of the NOA patients have hypogonadotropic hypogonadism.[7]

Ultrasonography is used for differentiation of obstructive azoospermia (AO) and NOA. Blood supply to testes is monitored by Doppler ultrasound.[8] For unilateral or bilateral absence of vas deferens ultrasonography was used.[9] Smaller testicular size with elevated FSH supported the diagnosis of NOA.[5,6] In case of normal size of testes and normal levels of FSH, LH and testosterone, testicular biopsy was recommended for the differentiation of OA from NOA.[10,11]

Chromosomal abnormalities in infertile men are about 12.70%.[12] It is observed that 11.70% are structural and 0.94% is numeric using karyotyping. The other main cause of infertility is microdeletion of the long arm Yq11 which is about 0.23%. This deletion is mainly associated with NOA presenting with spermatogenic failure and severe oligospermia.[13] Y chromosome microdeletion is the second most frequent genetic cause after Klinefelter syndrome. A locus located on Yq11 contains genes which have been involved in male infertility, called azoospermic factor (AZF) region.[14] This locus is divided into three major portions

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called AZFa, AZFb and AZFc.[15] Deletion in azoospermia (DAZ) gene is located within the AZFc region.[16] It was observed that in severe oligospermia deletions of the DAZ1/DAZ2 gene doubled. In different testicular phenotypes it is found that DAZ1/DAZ2 was involved, but later on it are reported that only DAZ1 is responsible for this phenotype.[17] Histopathological variations were observed in different AZF region deletions like Sertoli Cell Only Syndrome associated with AZFa region deletion while AZFb region was associated with maturation arrest in meiosis and AZFc microdeletion was found in hypospermatogenesis.[18-21]

To investigate the chromosomal aberrations and Y chromosome microdeletions in male infertile families

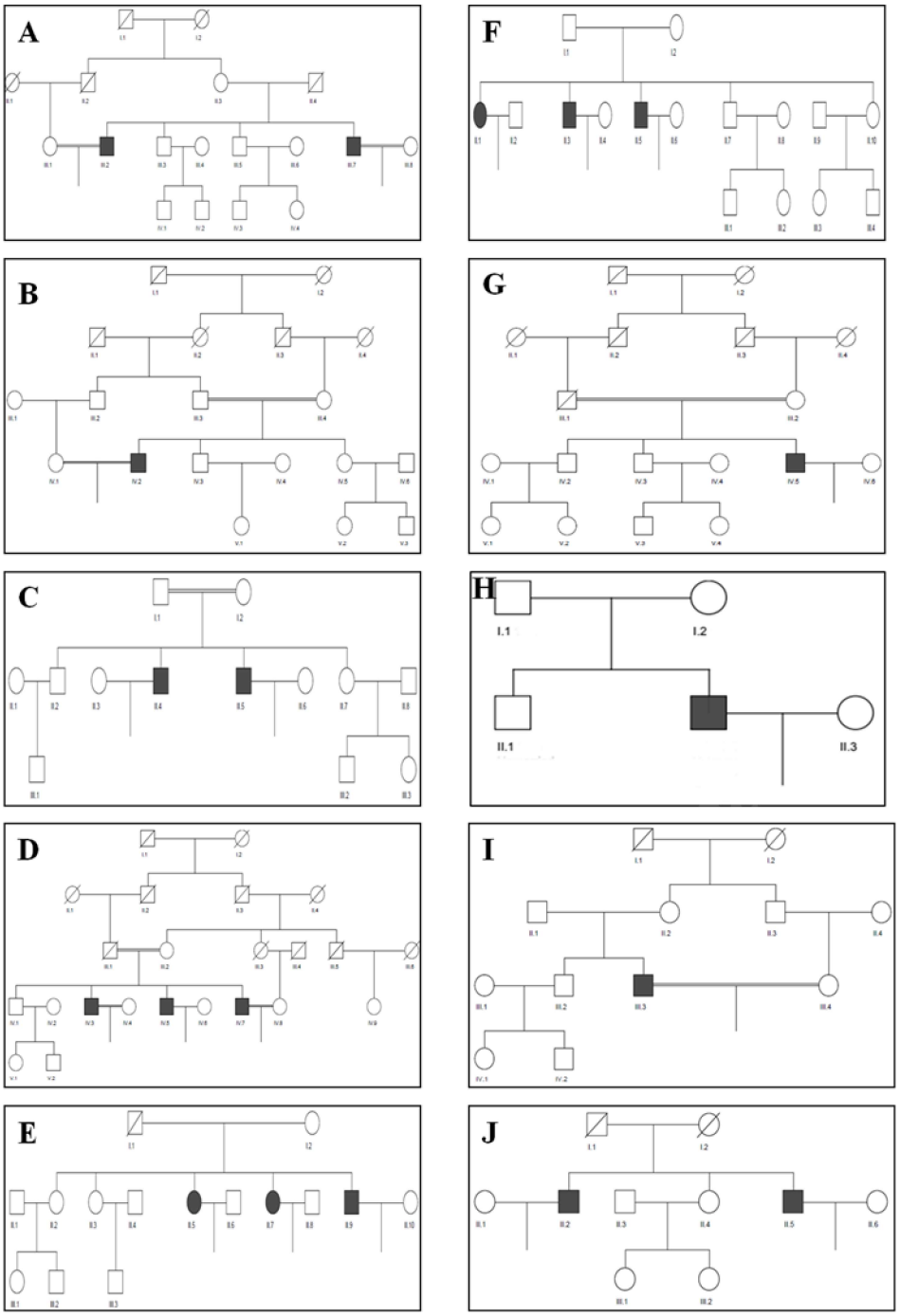

Figure : Pedigrees of families A,B,C,D,E,F,G,H,I and J. The affected individuals are shown with black filled square and circles. The diagonal line crossing indicates the deceased individuals. The double line indicates cousin marriages and the infertility is proven by no offspring with empty line.

Methods

The study was approved by institutional review board of Khyber Medical University Peshawar and is in accordance to Helsinki Declaration. Ten Pakistani families (A, B, C, D, E, F, G, H, I and J) were enrolled in this study (Figure). Informed consent was obtained from all participants of the family individually. Patients ages ranged from 18 to 45 years. Clinical evaluation was performed at local district hospital. All available male infertile members of ten families were evaluated as describe by Gudeloglu.[22] This study was conducted from January 2016 to October 2017.

Semen were collected and analysed according to WHO guidelines from all normal and affected participants of the study. Semen analyses were repeated after one month to confirm reproducibility of results.[1] The blood was collected in spray-coated silica and a polymer gel tubes (BD) and allowed to clot. Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH), Testosterone and Prolactin

were analysed from serum by chemiluminescence immunoassay Abbott architect.

To exclude the secondary cause of infertility Doppler ultrasonography was performed for all patients to assess testicular volume, blood supply, varicocele, hydrocele, vas deferens, cyst or any epididymis anomalies.[23] Testicular biopsies were taken and stained with haematoxylin and eosin to explore detailed microscopic structure of testes.[24]

Peripheral blood samples were collected from all patients into heparin test tube (BD) and cultured for 72 hours in RPMI-1640 medium supplemented with foetal bovine serum and phytohaemagglutinin. Cytogenetic analysis was performed for all patients using GTG banding technique.[25] At least 30 metaphases were analysed. The karyotypes were described according to the ISCN 95 nomenclature.[26]

Venous blood samples of 5ml in Ethylene diamine tetra acetic acid (BD) tubes were collected from all the subjects and were stored at 4-8°C until DNA extraction was carried out. The genomic DNA was extracted using the GentraPure Gene blood kit (Qiagen, USA) according to the manufacturer instructions. All the DNA samples were stored at -20°C. The concentration of DNA was quantified by Nano Drop 2000 spectrophotometer (Thermo scientific, USA) and purity was analysed by using Qubit 2.0 Fluorometer (Invitrogen, Life Technologies USA).

For AZFa, AZFb and AZFc regions of the Y chromosomes, PCR reactions were performed by using sY14, sY86, sY127 and sY254 regions primers, respectively. The amplified products were resolved on 2% agarose gel and the results were recorded and interpreted.[27]

Results

Family A has two affected members with severe oligoasthenozoospermia. Proband III-2 has normal FSH, LH and prolactin with low testosterone levels while proband III-7 has hypergonadotrophic hypogonadism with hyperprolactenaemia (Table 1).

Family C has two azoospermic patients with hypergonadotrophic hypogonadism. Proband III-4 has hyper prolactenaemia with normal testosterone while proband III-5 has low prolactin and testosterone (Table 1).

Family D has three affected members. Proband IV-3 and IV-5 has normal FSH, LH and prolactin with low testosterone levels (Table 1). In seminal parameters, only low motility was observed in both probands. Proband IV-7 has hypergonatrophic hypogonadism with low testosterone levels. Oligoasthenozoospermia was observed on semen analysis.

Table-1: Hormones and cytogenetics analysis of the families.

Family	ID	Karyotype	FSH	LH	Testosterone	Prolactin
A	III-2	Normal	11.1	9.07	6.81	140
	III-7	Normal	17.46	12.48	691	757.5
B	IV-2	Normal	30.1	16.7	21.3	322
C	III-4	Normal	16.71	11.56	371	455.3
	III-5	Normal	15.98	11.23	5.9	8.2
D	IV-3	Normal	5.26	2.15	6.19	208
	IV-5	Normal	10.3	8.51	5.16	202
	IV-7	Normal	65.3	7.75	4.91	157
E	II-9	Normal	2.85	3.41	4.71	108
F	II-3	Normal	8.97	4.59	266.4	188.3
	II-5	Normal	3.94	3.55	411.4	161.2
G	IV-5	0X	16.30	19.80	1.43	8.9
H	II-2	Normal	25	25.4	8.8	39.8
I	III-3	XXY	45.17	25.31	8.7	170.4
J	II-2	Normal	8.20	11.29	4.89	No data
	II-5	Normal	4.34	No data	No data	No data

Family E has one affected member with normal hormonal analysis (Table 1). On seminal analysis oligoasthenoteratozoospermia was observed.

Family F has two oligoasthenoteratozoospermic patients with normal hormonal study. Family G has one azoospermic member with hypergonadotrophic hypogonadism. Low testosterone was observed on hormonal analysis. Family H and I have one azoospermic member each. On hormonal analysis, low testosterone level with hypergonadotrophic hypogonadism was observed in both patients (Table 1). Family J has two azoospermic members (II-2, II-5). They have normal hormonal analysis (Table 1).

Family A proband III-2 has normal ultrasonography findings while proband III-7 has slight atrophy of testes bilaterally. In family C both probands have bilateral testicular atrophy. In family D proband IV-3 and IV-5 has normal ultrasound while proband IV-7 has left testicular atrophy. Affected member in family E has normal ultrasound study. Family F has two probands, one II-3 has Varicocele observed on ultrasonography while proband II-5 has normal ultrasound study. Family G, H and I have one affected member each with bilateral atrophic testis.

Only two families revealed numerical anomalies. Family G has one azoospermic member with 0X karyotype while

Table-2: Ultrasonographic and Y chromosome microdeletion analysis of the families.

Family	ID	Testicular atrophy	Varicocele	Hernia	Vas deferens	AZFa	AZFb	AZFc
A	III-2	Normal	Absent	Absent	Palpable	Present	Present	Present
	III-7	Normal	Absent	Absent	Palpable	Present	Present	Present
B	IV-2	Bilateral	Absent	Present	Palpable	Present	Present	Present
C	III-4	Bilateral	Absent	Absent	Palpable	Present	Present	Deleted
	III-5	Bilateral	Absent	Absent	Palpable	Present	Present	Deleted
D	IV-3	Left testes	Absent	Absent	Palpable	Present	Present	Present
	IV-5	Normal	Absent	Absent	Palpable	Present	Present	Present
E	IV-7	Normal	Absent	Absent	Palpable	Present	Present	Present
F	II-9	Normal	Present	Absent	Palpable	Present	Present	Present
	II-3	Normal	Present	Absent	Palpable	Present	Present	Present
G	IV-5	Bilateral	Absent	Absent	Palpable	Present	Present	Present
H	II-2	Bilateral	Absent	Absent	Palpable	Present	Present	Present
I	III-3	Bilateral	Absent	Absent	Palpable	Present	Present	Present
J	II-2	Bilateral	Absent	Absent	Palpable	Present	Present	Present

family I had azoospermic member with XXY karyotype on cytogenetic analysis. Families A, B, C, D, E, F, H and J were found normal for both structural and numerical anomalies (Table 2).

The Y chromosome microdeletion analysis was revealed only in family C with deletion of AZFc region in two azoospermic affected (III-4, III-5) participants of the study. It was observed that 10% of azoospermia is due to Y chromosome microdeletion and especially AZFc region deletion. Chromosomal abnormalities contributed 20% of azoospermia in Khyber Pakhtunkhwa population. Abnormal fertility hormone analysis i.e FSH, LH, testosterone and testicular ultrasonography was observed in six male infertile families. These findings clearly indicate that hormone analysis, scrotal ultrasound, karyotyping and Y chromosome microdeletion have major role in the diagnosis of male infertility.

Discussion

The frequency of AZFc microdeletion is about 10% in the present study. Family C has two azoospermic males. Both members showed AZFc deletion on PCR analysis, which is congruence to 10-15% AZF microdeletion in azoospermic patients and severe oligospermic patients as reported somewhere else.[28] Another study has reported 5.06% frequency of AZF deletion in 3654 patients.[29] About 60% of the families have recorded atrophic testis on doppler ultrasound. In these patients hypergonadotrophic hypogonadism was observed. In another study, it was observed that patients with AZFc deletions have mild spermatogenic disruption. In some tubules, germ cells were observed in different developmental stages.[18] The primary cause of spermatogenic failure observed in AZFc deletion is due to the sperm maturation defect or post meiotic spermatid defect. In the AZFc region, partial deletion of DAZ gene caused hypospermatogenesis.[30] In our study one family of AZFc deletion has high levels of FSH and LH with atrophic testis. Liu et al also reported that in the AZF regions deletion patients have high level of FSH.[31] About 60% families observed high FSH and LH levels. These families also showed atrophic testis. According to Tureks if a patient has high value of FSH and LH with atrophic testis; this patient has NOA. Therefore it can be concluded that 60% families investigated in the current study have NOA.

Conclusion

It is concluded from this study that about 10% male families were affected from Y chromosome microdeletions. About 20% families were affected from chromosomal abnormalities. Fertility hormone and scrotal ultrasound were altered in 60% male infertility cases. In the routine investigation of male infertility, semen analysis, scrotal ultrasound and fertility hormone FSH, LH and testosterone are to be included. The occurrence of Y chromosome microdeletions among infertile males strongly suggests the need of Y chromosome microdeletion to include in routine tests to investigate the causes of male infertility.

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