RESEARCH ARTICLE

Role of Genetics in Human Infertility

Ronak V Manikawala[1], Jigna Desai[2]

Received on: 27 November 2022; *Accepted on:* 20 November 2023; *Published on:* xxxx

Abstract

Aim: To study the role of genetics in human infertility.

Materials and methods: We collected 60 male and 41 female blood samples from infertile couples and carried out a cytogenetic study in both males and females, whereas molecular study was done in only male subjects. Standard Giemsa banding karyotyping protocol was followed for the cytogenetic study. For azoospermia factor (AZF) microdeletion studies, deoxyribonucleic acid (DNA) isolation was followed by multiplex polymerase chain reaction (PCR) using AZF-specific sequence tagged site (STS) markers.

Results: In the female cytogenetic study, no structural or chromosomal abnormalities were found; three (7.31%) had polymorphic variants. In males, one (1.6%) had autosomal structural 46, XY, t (3; 17) (p25; q22), one (1.6%) had sex chromosomal numerical abnormalities 47, XXY (12)/46, XX (8) and four (6.66%) had the polymorphic variant. Two males and two females had 9qh+ with other normal chromosomal constitutions. In the same way, two other types of polymorphism were also observed, that is, 21pS+ in one male and one female, whereas 22pS+ was observed in one male subject. The translocation 46, XY, t (3; 17) (p25; q22) we have found is unique. In male blood samples, we studied six gene mutations named *AZFa (sy84)*, *AZFa (sy86)*, *AZFb (sy127)*, *AZFb (sy134)*, *AZFc (sy254)*, and *AZFc (sy255)*. We have observed deletions in eight subjects with a microdeletion frequency of 13.33%, where seven (87.5%) were azoospermic, and one (12.5%) was oligozoospermic. Most found microdeletions were *AZFb (sy127)* in three males and *AZFb (sy134)* in three males. *AZFa (sy86)* and *AZFa (sy254)* deletions were found in one male.

Conclusion: As per our knowledge, there is one novel translocation 46, XY, t (3; 17) (p25; q22) in one male patient from our research. We have also found AZF microdeletions in oligozoospermic and azoospermic patients. The results indicate the importance of karyotyping and microdeletion screening in chromosome Y for infertile couples before advising them of costlier treatments.

Clinical significance: The present investigation is a valuable consideration for prognosis, which can be helpful for counseling couples and minimizing the potential risk of transmission of genetic abnormalities to future generations.

Keywords: Azoospermia factor microdeletions, Infertility, Karyotyping.

International Journal of Infertility and Fetal Medicine (2023): 10.5005/jp-journals-10016-1329

Introduction

Infertility is now a major social concern as it affects couples in many ways. For infertility, many of the risk factors are shared by both males and females. Which include tobacco, smoking, age, use of alcohol, too much exercise, and caffeine intake.[1] For female infertility, identifiable factors include endocrine or hormonal disturbances, tubal factors, acquired nontubal factors, congenital abnormalities, and sexual dysfunction. For male infertility, various reasons can be accounted, like abnormalities in sperm, formation of antisperm antibodies, varicocele, ejaculatory duct obstruction, hormonal imbalance, environmental factors, chromosomal aberrations, and azoospermia factor (AZF) microdeletions.[2] Idiopathic infertility can be seen in abnormal semen analysis resulting without etiological factors identifiable from history or physical examination.[3] Around 30% of male infertility cases are assumed that they are caused by genetic mutations of the male germ line or chromosomal abnormalities. Infertility causes of infertility are classified into three groups: chromosomal aneuploidies, submicroscopic deletions, and single-gene defects.

These days, karyotyping has become an essential examination in the diagnostics system of any infertile male or female. Interstitial deletions or single gene defects in deoxyribonucleic acid (DNA) are the other most common abnormalities. Some translocations are very rare involving Y and an autosomal chromosome. The most frequent translocation is a translocation between the heterochromatin region in the Y-chromosome's long arm (breakpoint at q12) and the short arm of chromosome 15 (breakpoint at p11–13). From sequence homology, this can be the outcome of a recurrent alliance at the Yq heterochromatin and pachytene stage of male meiosis of the 15p.[4] Y-chromosome microdeletion (YCM) is a genetic disorder evoked by missing genes in the Y-chromosome. Several males live a normal life without any symptoms exhibiting YCM. However, a vital number of males with decreased fertility are noted to have YCM.[5] There is a 15–20% YCMs incidence found in azoospermia men being idiopathic, in which severe oligozoospermia men are found to be 7–10%. Recurrent YCM define three nonoverlapping regions of Yq, termed AZF, where AZFa has been associated with Sertoli cell-only (SCO) syndrome, AZFb is associated with spermatogenic arrest, and AZFc is associated with variable phenotype (severe oligozoospermia or azoospermia).[6] Many studies support the general trend that failure of spermatogenesis is related to deletions of the long arm of the Y-chromosome; still, phenotype/genotype correlations are difficult to establish.[7]

[1,2]Department of Biosciences, Veer Narmad South Gujarat University, Surat, Gujarat, India

Corresponding Author: Ronak V Manikawala, Department of Biosciences, Veer Narmad South Gujarat University, Surat, Gujarat, India, Phone: +91 9426541311, e-mail: ronak_manika@yahoo.com

How to cite this article: Manikawala RV, Desai J. Role of Genetics in Human Infertility. Int J Infertil Fetal Med 2023;https://doi.org/10.5005/jp-journals-10016-1329.

Source of support: Nil

Conflict of interest: None

Materials and Methods

The sample collection was started in June 2021. The research work is being carried out at SN GeneLab, Surat, Gujarat, India. The study involved 60 infertile males and 41 infertile females for genetic analysis, with 60 male controls and 30 female controls. The work started by meeting with gynecologists and *in vitro* fertilization (IVF) specialists to understand the infertility problems, diagnosis, treatment, study pattern, and outcome of the study. A complete proposal and detailed methodology were put forward to the Institutional Ethical Committee (IEC) for approval. After IEC was approved, a meeting was arranged with the gynecologists for permission for blood collection from infertile patients. Regarding the confidentiality of the subject, a unique code IM-1 and IM-2 for male blood samples and an IF-1 and IF-2 code for female blood samples were used to avoid the usage of the patient's name for further research and report generation. Written consent was taken from patients. A total of 15 (36.58%) females were >30 years of age. A total of 26 (63.41%) females were <29 years of age. Out of 41 subjects, 24 (58.53%) females had one or more than one abortion, whereas 17 (41.46%) females had never conceived. Only two females had irregular menstrual history.

The peripheral blood lymphocyte culture technique was used for metaphase preparation. The Standard procedure of Hungerford was followed with slight modifications.[8] Giemsa banding was done following the protocol described by Seabright.[9] A minimum of 20 metaphases were examined from cultures of peripheral blood for each patient, whereas in the case of mosaicism, 100 metaphases were analyzed. Ikaros karyotyping software was used for metaphase analysis. For mutation analysis, DNA isolation was done using the phenol-chloroform method.[10] The male patients' DNA was routed to multiplex polymerase chain reaction (PCR) using AZF-specific sequence tagged site (STS) markers. A pair of each primer replicates a particular region of the AZF locus located in the long arm of the Y-chromosome. As an internal control, the zinc finger Y-chromosomal protein (ZFY) gene was used, whereas the SRY gene was examined to validate the gender of the donor. The reactions were set using forward and reverse primers for all AZF genes. The total reaction mixture per tube was kept at 25 µL with standard mastermix preparation.

Primer Sequences for AZF Gene

- *AZFa (sy84)* (BP: 326).
  - F: 5'AGAAGGGTCTGAAAGCAG GT 3'.
  - R: 5'GCCTACTACCTGGAGGCTTC 3'.
- *AZFa (sy86)* (BP:320).
  - F: 5' - GTG ACA CAC AGA CTA TGC TTC–3'.
  - R: 5' - ACA CAC AGA GGG ACA ACC CT–3'.
- *AZFb (sy127)* (BP:274).
  - F: 5'GGCTCACAAACGAAAAGAAAA 3'.
  - R: 5'CTGCAGGCAGTAATAAGGGA 3'.
- *AZFb (sy134)* (BP:301).
  - F: 5'GTCTGCCTCACCATAAAACG 3'.
  - R: 5'ACCACTGCCAAAACTTTCAA 3'.
- *AZFc (sy254)* (BP: 400).
  - F: 5'GGGTGTTACCAGAAG GCAAAA 3'.
  - R: 5' GAACCGTATCTACCAAAG CAG C 3'.
- *AZFc (sy255)* (BP:126).
  - F: 5' GTT ACA GGA TTC GGC GTG AT 3'.
  - R: 5' CTC GTC ATG TGC AGC CAC 3'.
- ZFY (BP:495).
  - F: 5' ACC RCT GTA CTG ACT GTG ATT ACA C 3'.
  - R: 5' GCA CYT CTT TGG TAT CYG AGA AAG T 3'.
- SRY (BP: 472).
  - F: 5' GAA TAT TCC CGC TCT CCG GA 3'.
  - R: 5' GCT GGT GCT CCA TTC TTG AG 3'.

The reaction tubes were placed in a thermal cycler and subjected to initial denaturation, 35 cycles of Initial denaturation (95°C, 5 minutes), denaturation (95°C, 30 seconds), annealing of primers (55°C, 30 seconds), extension (72°C, 30 seconds) and polymerization followed by final extension step (72°C, 5 minutes). The PCR products were analyzed by agarose gel electrophoresis.

Results

The cytogenetic study was carried out with a total of 41 female subjects and 60 male subjects. All female subjects showed normal karyotypes, and only three (7.31%) had polymorphic variants at 450–500 band resolution. Out of 60 male subjects, 40% were oligozoospermic, and 60% were azoospermic. A total of 58 males had normal karyotypes, four had polymorphic variants in which three subjects were azoospermic, and one was oligozoospermic. In one other oligozoospermic subject, structural abnormality, that is, 46, XY, t (3; 17), was found. One mosaic numerical abnormality 47, XXY (12) /46, XX (8) was found in the other azoospermic subject. Their karyotype results are shown in Tables 1 and 2. The image of chromosomal aberration is shown in Figure 1.

In 60 male subjects, AZF microdeletion studies were also done along with cytogenetic findings. Here, we identified deletions in eight patients with a microdeletion frequency of 13.33% (Table 3). Among these, seven subjects (87.5%) were azoospermic, and one (12.5%) subject was oligozoospermic. We did not find *AZFa (sy84)* and *AZFc (sy255)* deletion in any patient. Three subjects had *AZFb (sy127)*, three subjects had *AZFb (sy134)*, one subject had *AZFa (sy86)*, and one subject had *AZFc (sy254)* deletions. The subject with *AZFc (sy254)* deletion was oligozoospermic, and the rest of the deletions found in all subjects were azoospermic.

Discussion

The latest findings have focused on genetic factors responsible for infertility in both males and females despite the known prevalence of infertility. It is assumed that, in approximately 15% male and 10% female infertile subjects, chromosome aberrations or single gene mutations could be present.[11]

With the recent trend toward delaying childbearing, natural age limits of fertility in both males and females have become more evident. Normally, 75% of women try to conceive at 30 years of age, and they can get a conception ending in a live birth within a year, 66% at age 35 years, and 44% at age 40 years.[12] The present study includes 15 (36.58%) females who were 30 or >30 years of age. A total of 26 (63.41%) females were 29 or <29 years old (Table 4).

The most common complication of pregnancy is spontaneous abortion, which affects roughly one in four of all pregnant women.[13] The causes of miscarriage are often unknown. In our study of 41 subjects, 24 (58.53%) females had one or more than one abortion, whereas 17 (41.46%) females had never conceived (Table 4).

Here in this study, a total of 40% of males were oligozoospermic, and 60% were azoospermic (Table 5).

The existence of abnormalities in chromosomes is higher in infertile males, which is inversely related to the sperm count. On the

Table 1: Karyotype results of female subjects

Sample no.	*Result*
IF-1	46, XX
IF-2	46, XX
IF-3	46, XX
IF-4	46, XX
IF-5	46, XX
IF-6	46, XX
IF-7	46, XX
IF-8	46, XX
IF-9	46, XX
IF-10	46, XX
IF-11	46, XX
IF-12	46, XX
IF-13	46, XX
IF-14	46, XX
IF-15	46, XX
IF-16	46, XX
IF-17	46, XX
IF-18	46, XX
IF-19	46, XX
IF-20	46, XX
IF-21	46, XX
IF-22	46, XX
IF-23	46, XX
IF-24	46, XX
IF-25	46, XX
IF-26	46, XX
IF-27	46, XX, 9qh+
IF-28	46, XX
IF-29	46, XX
IF-30	46, XX, 21pS+
IF-31	46, XX
IF-32	46, XX
IF-33	46, XX
IF-34	46, XX
IF-35	46, XX
IF-36	46, XX
IF-37	46, XX
IF-38	46, XX
IF-39	46, XX
IF-40	46, XX, 9qh+
IF-41	46, XX

Table 2: Karyotype results of male subjects

Sample no.	*Result*
IM-1	46, XY
IM-2	46, XY, t (3; 17) (p25; q22)
IM-3	46, XY
IM-4	46, XY
IM-5	46, XY
IM-6	46, XY
IM-7	46, XY
IM-8	46, XY
IM-9	46, XY
IM-10	46, XY
IM-11	46, XY
IM-12	46, XY
IM-13	46, XY
IM-14	46, XY
IM-15	46, XY
IM-16	46, XY
IM-17	46, XY
IM-18	46, XY
IM-19	46, XY
IM-20	46, XY
IM-21	46, XY
IM-22	46, XY
IM-23	47, XXY (12)/46, XX (8)
IM-24	46, XY
IM-25	46, XY
IM-26	46, XY
IM-27	46, XY
IM-28	46, XY
IM-29	46, XY, 22pS+
IM-30	46, XY, 9qh+
IM-31	46, XY
IM-32	46, XY
IM-33	46, XY
IM-34	46, XY
IM-35	46, XY
IM-36	46, XY
IM-37	46, XY
IM-38	46, XY
IM-39	46, XY
IM-40	46, XY
IM-41	46, XY
IM-42	46, XY
IM-43	46, XY
IM-44	46, XY
IM-45	46, XY
IM-46	46, XY
IM-47	46, XY
IM-48	46, XY
IM-49	46, XY
IM-50	46, XY
IM-51	46, XY, 9qh+
IM-52	46, XY
IM-53	46, XY, 21pS+
IM-54	46, XY
IM-55	46, XY
IM-56	46, XY
IM-57	46, XY
IM-58	46, XY
IM-59	46, XY
IM-60	46, XY

basis of most of the research, it has been estimated that the general incidence of a chromosomal factor in infertile men lies between 2 and 8%, with a mean value of 5%,[11] whereas the estimation of chromosomal abnormalities in women is 10.0%.[14]

In the present research, no structural or chromosomal abnormalities were found in females; three (7.31%) had polymorphic variants (Table 1).

In males, one (1.6%) had autosomal structural, one (1.6%) had sex chromosomal numerical abnormalities, and four (6.66%) had polymorphic variants (Table 2).

The commonest type of karyotype abnormality is Klinefelter's syndrome (KS), detected in infertile subjects.[11] In the current investigation, one male (IM:23) had a mosaic chromosomal constitution, that is, mos 47, XXY (12)/46, XX (8).

Along with chromosomal abnormalities, chromosomal polymorphism is identified often among infertile patients. Chromosomal polymorphisms can also affect both women's and men's infertility, although it has to be clarified in the publications.[15]

Zhang concluded that chromosome 1 and chromosome 3 are frequently intricated in balanced reciprocal translocations in the males of Northeastern China who were infertile or receiving genetic counseling.[16] They also found the 46, XY, t (3; 17) (q25; q23) in their study of 3,148 men, but 46, XY, t (3; 17) (p25; q22) translocation obtained in our case is not reported anywhere as per our knowledge (Fig. 1).

Chromosome 9 is understood to be extremely polymorphic among the nonacrocentric chromosomes with a high level of interchromosomal and intrachromosomal duplications. Variants due to regions of chromosome 9, like 9qh+, 9cenh+, 9ph+, 9qh−, or inv (9) (p11q13), known as "heteromorphisms" or "heterochromatic variants," are generally found with an overall frequency of more or less 1.5% in the general population.[17] In our research, two males (IM-30 and IM-51) and two females (IF-27 and IF-40) had 9qh+ with other normal chromosomal constitution. Same way, two other types of polymorphism were also observed, that is, 21pS+ in one male and one female, whereas 22pS+ was observed in one male subject (IM-29).

The frequent event seen in azoospermic and severe oligozoospermic patients is deletions in the AZF locus.[18] Etiology is idiopathic in roughly 30–50% of all cases of azoospermia or severe oligozoospermia.[19]

The percentage of infertile males with microdeletions in one or more subregions worldwide ranges from 0.7 to 34.5%, with an average of 8.2%.[20]

In our research, we studied six gene mutations named *AZFa (sy84)*, *AZFa (sy86)*, *AZFb (sy127)*, *AZFb (sy134)*, *AZFc (sy254)*, and *AZFc (sy255)*. We found deletions in eight patients with a frequency of 13.33%, where seven (87.5%) were azoospermic, and 1(12.5%) was oligozoospermic. We did not find *AZFa (sy84)* and *AZFc (sy255)* deletion in any patient. In contrast, *AZFa (sy86)* deletion was found in one male, and *AZFc (sy254)* deletion was found in one male. *AZFb (sy127)* and *AZFb (sy134)* deletion were found in three males.

More microdeletions were found in *AZFb (sy127)* and *AZFb (sy134)*. The limited sample size (60 patients) and rigid inclusion criteria of patients may lead to little increase in the frequency of deletion.

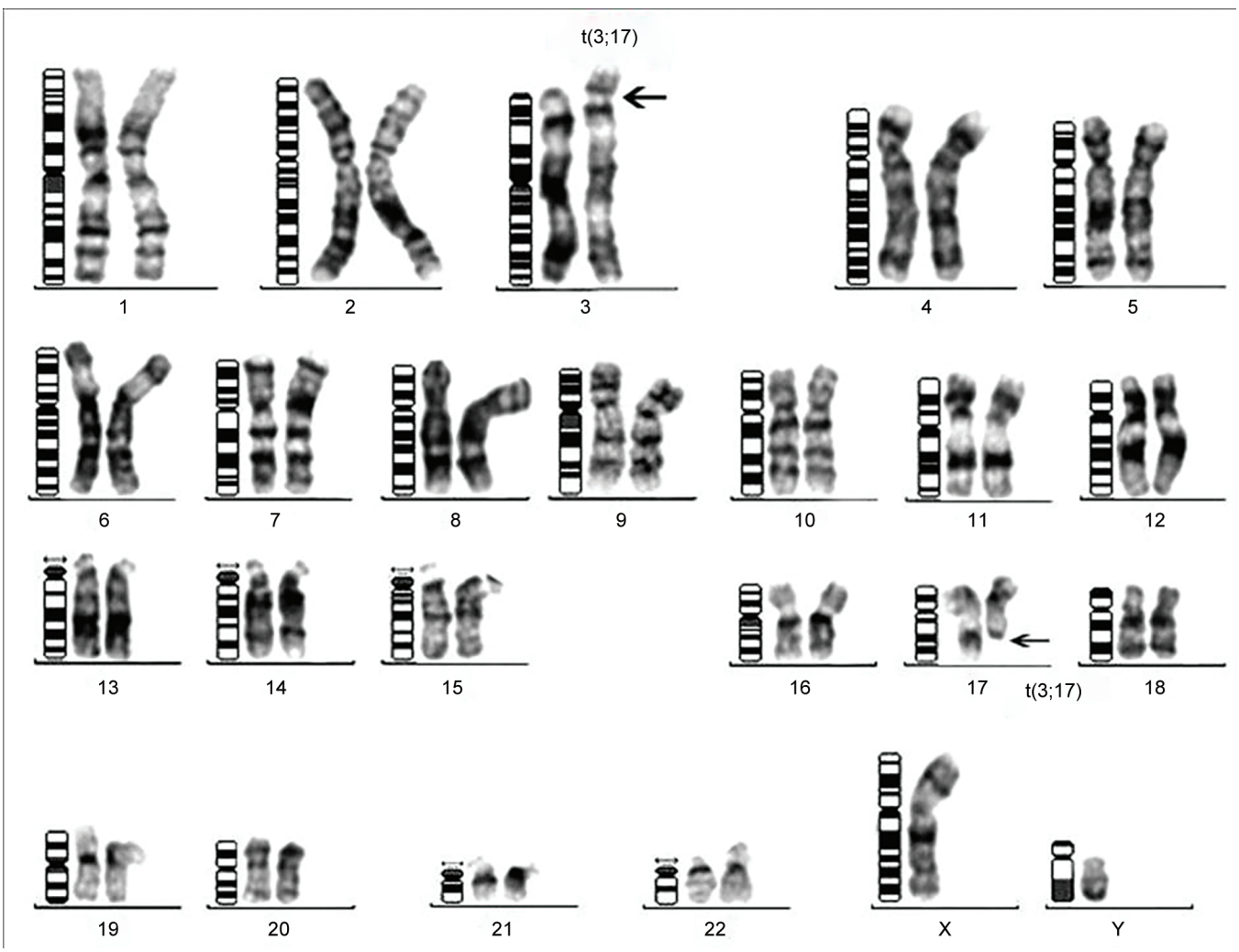

Fig. 1: Image of chromosomal aberration

Table 3: Azoospermia factor (AZF) gene microdeletion study (+ indicates the presence of a band, – indicates an absence of a band on agarose gel electrophoresis)

	SRY	*ZFY*	*AZFa (sy84)*	*AZFa (sy86)*	*AZFb (sy127)*	*AZFb (sy134)*	*AZFc (sy254)*	*AZFc (sy255)*
IM-01	+	+	+	+	+	+	+	+
IM-02	+	+	+	+	+	+	+	+
IM-03	+	+	+	–	+	+	+	+
IM-04	+	+	+	+	+	+	+	+
IM-05	+	+	+	+	+	+	+	+
IM-06	+	+	+	+	+	+	+	+
IM-07	+	+	+	+	+	+	+	+
IM-08	+	+	+	+	+	+	+	+
IM-09	+	+	+	+	+	+	+	+
IM-10	+	+	+	+	+	+	+	+
IM-11	+	+	+	+	+	–	+	+
IM-12	+	+	+	+	+	+	+	+
IM-13	+	+	+	+	+	+	+	+
IM-14	+	+	+	+	+	+	+	+
IM-15	+	+	+	+	+	+	+	+
IM-16	+	+	+	+	+	+	+	+
IM-17	+	+	+	+	+	+	+	+
IM-18	+	+	+	+	+	+	+	+
IM-19	+	+	+	+	+	+	+	+
IM-20	+	+	+	+	+	+	+	+
IM-21	+	+	+	+	+	–	+	+
IM-22	+	+	+	+	+	+	+	+
IM-23	+	+	+	+	+	+	+	+
IM-24	+	+	+	+	+	+	+	+
IM-25	+	+	+	+	+	+	+	+
IM-26	+	+	+	+	–	+	+	+
IM-27	+	+	+	+	+	+	+	+
IM-28	+	+	+	+	+	+	+	+
IM-29	+	+	+	+	–	+	+	+
IM-30	+	+	+	+	+	+	+	+
IM-31	+	+	+	+	+	+	+	+
IM-32	+	+	+	+	+	+	+	+
IM-33	+	+	+	+	+	+	+	+
IM-34	+	+	+	+	+	+	+	+
IM-35	+	+	+	+	+	+	+	+
IM-36	+	+	+	+	+	+	–	+
IM-37	+	+	+	+	+	+	+	+
IM-38	+	+	+	+	+	+	+	+
IM-39	+	+	+	+	+	+	+	+
IM-40	+	+	+	+	+	+	+	+
IM-41	+	+	+	+	+	+	+	+
IM-42	+	+	+	+	+	+	+	+
IM-43	+	+	+	+	+	+	+	+
IM-44	+	+	+	+	+	+	+	+
IM-45	+	+	+	+	–	+	+	+
IM-46	+	+	+	+	+	+	+	+
IM-47	+	+	+	+	+	+	+	+
IM-48	+	+	+	+	+	+	+	+
IM-49	+	+	+	+	+	+	+	+

Contd…

Contd…

	SRY	*ZFY*	*AZFa (sy84)*	*AZFa (sy86)*	*AZFb (sy127)*	*AZFb (sy134)*	*AZFc (sy254)*	*AZFc (sy255)*
IM-50	+	+	+	+	+	+	+	+
IM-51	+	+	+	+	+	+	+	+
IM-52	+	+	+	+	+	+	+	+
IM-53	+	+	+	+	+	+	+	+
IM-54	+	+	+	+	+	+	+	+
IM-55	+	+	+	+	+	–	+	+
IM-56	+	+	+	+	+	+	+	+
IM-57	+	+	+	+	+	+	+	+
IM-58	+	+	+	+	+	+	+	+
IM-59	+	+	+	+	+	+	+	+
IM-60	+	+	+	+	+	+	+	+

Table 4: Compiled data from pro forma of female subjects who participated in the study

Sample no.	*Age/sex*	*History*	*Menstrual history*	*Marriage life (years)*
IF-1	26 years/female	Two abortions	Regular	2
IF-2	27 years/female	One abortion	Regular	3
IF-3	27 years/female	Two abortions	Regular	5
IF-4	32 years/female	Two abortions	Regular	6
IF-5	23 years/female	Two abortions	Regular	3
IF-6	29 years/female	Infertility	Irregular	5
IF-7	30 years/female	Two abortions	Regular	7
IF-8	34 years/female	Two abortions	Regular	13
IF-9	33 years/female	Infertility	Regular	9
IF-10	27 years/female	Infertility	Regular	2
IF-11	29 years/female	Four abortions	Regular	3
IF-12	27 years/female	Four abortions	Regular	5
IF-13	25 years/female	Infertility	Regular	3
IF-14	29 years/female	Two abortions	Regular	5
IF-15	31 years/female	Two abortions	Regular	9
IF-16	30 years/female	Infertility	Irregular	7
IF-17	26 years/female	Infertility	Regular	2
IF-18	27 years/female	Infertility	Regular	3
IF-19	29 years/female	Two abortions	Regular	6
IF-20	32 years/female	Two abortions	Regular	3
IF-21	27 years/female	Three abortions	Regular	5
IF-22	32 years/ female	Infertility	Regular	7
IF-23	38 years/female	Infertility	Regular	14
IF-24	29 years/female	Infertility	Regular	6
IF-25	30 years/female	Infertility	Regular	4
IF-26	25 years/female	Infertility	Regular	4
IF-27	33 years/female	Three abortions	Regular	12
IF-28	27 years/female	Two abortions	Regular	3
IF-29	29 years/female	Infertility	Regular	7
IF-30	28 years/female	Two abortions	Regular	4
IF-31	31 years/female	Infertility	Regular	7
IF-32	23 years/female	Three abortions	Regular	3
IF-33	27 years/female	Two abortions	Regular	5
IF-34	29 years/female	Two abortions	Regular	4
IF-35	26 years/female	Infertility	Regular	5
IF-36	29 years/female	Four abortions	Regular	8
IF-37	28 years/female	Infertility	Regular	4
IF-38	32 years/female	Two abortions	Regular	9
IF-39	30 years/female	Three abortions	Regular	8
IF-40	34 years/female	Three abortions	Regular	7
IF-41	27 years/female	Infertility	Regular	6

Table 5: Compiled data from pro forma of male subjects who participated in the study

Sample no.	*Age/sex*	*Sperm count (million/mL)*	*Marriage life (years)*
IM-1	30 years/male	11	5
IM-2	33 years/male	5	3
IM-3	27 years/male	0	4
IM-4	32 years/male	0	7
IM-5	25 years/male	0	3
IM-6	40 years/male	16	12
IM-7	31 years/Male	15	6
IM-8	28 years/male	0	4
IM-9	34 years/male	0	6
IM-10	27 years/male	7	5
IM-11	29 years/male	0	5
IM-12	33 years/male	9	3
IM-13	32 years/male	0	4
IM-14	26 years/male	0	3
IM-15	37 years/male	12	9
IM-16	33 years/male	0	8
IM-17	28 years/male	0	3
IM-18	32 years/male	8	3
IM-19	26 years/male	15	3.5
IM-20	32 years/male	0	3
IM-21	36 years/male	0	8
IM-22	30 years/male	0	7
IM-23	32 years/male	0	9
IM-24	36 years/male	3	5
IM-25	30 years/male	1	4
IM-26	34 years/male	0	7.5
IM-27	41 years/male	0	12
IM-28	30 years/male	9	3
IM-29	29 years/male	0	7
IM-30	29 years/male	0	4
IM-31	32 years/male	7	9
IM-32	29 years/male	0	6
IM-33	28 years/male	5	5
IM-34	33 years/male	4	4
IM-35	27 years/male	0	3
IM-36	33 years/male	15	9
IM-37	34 years/male	2	5
IM-38	26 years/male	6	3
IM-39	29 years/male	0	7
IM-40	31 years/male	0	6
IM-41	31 years/male	0	6
IM-42	28 years/male	0	3.5
IM-43	40 years/male	0	7
IM-44	42 years/male	17	8
IM-45	39 years/male	0	6.5
IM-46	27 years/male	1	3
IM-47	29 years/male	0	2
IM-48	31 years/male	0	2.5
IM-49	36 years/male	9	4
IM-50	40 years/male	0	4.5

Contd…

Contd…

Sample no	*Age/sex*	*Sperm count (million/mL)*	*Marriage life (years)*
IM-51	29 years/Male	0	3
IM-52	29 years/male	0	3.5
IM-53	30 years/male	7	4
IM-54	31 years/male	2	4
IM-55	33 years/male	0	5
IM-56	34 years/male	0	6
IM-57	35 years/male	0	6
IM-58	41 years/male	8	8
IM-59	42 years/male	0	9
IM-60	30 years/male	0	4

For the spermatogenic disruption, AZFa deletions are identified in SCO syndrome. The whole deletion of the AZFa locus eliminates 792 kb, which includes the two candidate genes USP9Y and DBY.[21]

Deletions in the AZFb gene are related to the complete absence of elongating spermatids and spermatozoa or SCOS.[22–24]

Large amounts of AZFc deletions decrease sperm density and show variable phenotypical characteristics ranging from azoospermia to mild or severe oligozoospermia.[25] These results are consistent with the genotype and phenotype correlation observed in other research. Microdeletion in AZFa or AZFb regions of the Y-chromosome denotes a remarkably poor prognosis for sperm retrieval, whereas most of the males with AZFc deletion have sperm within the semen or testes available for use in IVF/intracytoplasmic sperm injection.[26]

A similar type of study was done by Mafra et al. in Brazilian infertile men with severe oligozoospermia or nonobstructive azoospermia, and they were attending an infertility treatment. Abnormalities in genes were found in 18.8% of the undertaken patients. Chromosomal abnormalities were identified in 6.2% of the patients, being more recognized in the azoospermia group (11.6%) than in the oligozoospermia group (4%). Chromosomal variants were observed in 8.3%, and YCMs in 4.2% of patients.[27]

So, overall, the results gathered in our research fulfill the aim to analyze if there is a role of genetics in infertility, which can provide clinical significance, especially when there is idiopathic infertility.

Conclusion

One translocation and one numerical abnormality obtained in male subjects' karyotypes in our research indicate the importance of cytogenetic findings for infertility in both males and females. 46, XY, t (3; 17) (p25; q22). Translocation is a novel one obtained in our case as it is not reported anywhere as far as we know. AZFb deletion was found more frequently than other deletions; AZFc deletion was also found in subjects with oligo and azoospermia, estimating that YCMs contribute to a smaller number of idiopathic azoospermia and oligozoospermia leading to infertility. Further research in large populations is required to confirm the correlation of these genes with idiopathic infertility. The present investigation is a valuable consideration for prognosis, which can be helpful for counseling couples and minimizing the pitfall of transmission of genetic abnormalities to the next generations.

References

1. Dechanet C, Anahory T, Mathieu Daude JC, et al. Effects of cigarette smoking on reproduction. Hum Reprod Update 2011;17(1):76–95. DOI: 10.1093/humupd/dmq033
2. Natarajamani S, Janani D, Subramanian M, et al. Correlation of semen PH with other semen parameters in a sub fertile male population attending a tertiary ART center in South India. Int J Sci Res Pub 2014;4(8):355–359.
3. Vogt PH. Molecular basis of male infertility. Int J Androl 1997 20(Suppl 3):2–10. PMID: 9466180.
4. Onrat ST, Söylemez Z, Elmas M. 46, XX, der (15), t (Y; 15)(q12; p11) karyotype in an azoospermic male. Indian J Hum Genet 2012;18(2):241–245. DOI: 10.4103/0971-6866.100785
5. Foresta C, Ferlin A, Garolla A, et al. High frequency of well-defined Y-chromosome deletions in idiopathic Sertoli cell-only syndrome. Hum Reprod 1998;13(2):302–307. DOI: 10.1093/humrep/13.2.302
6. Vogt P, Chandley AC, Hargreave TB, et al. Microdeletions in interval 6 of the Y chromosome of males with idiopathic sterility point to disruption of AZF, a human spermatogenesis gene. Hum Genet 1992;89(5):491–496. DOI: 10.1007/BF00219172
7. Krausz C, McElreavey K. Y chromosome and male infertility. Front Biosci 1999;4:E1–E8. DOI: 10.2741/krausz
8. Hungerford DA. Leukocytes cultured from small inocula of whole blood and the preparation of metaphase chromosomes by treatment with hypotonic KCl. Stain Technol 1965;40(6):333–338. DOI: 10.3109/10520296509116440
9. Seabright M. A rapid banding technique for human chromosomes. Lancet 1971;2(7731):971–972. DOI: 10.1016/s0140-6736(71)90287-x
10. Lázaro-Silva DN, De Mattos JCP, Castro H, et al. The use of DNA extraction for molecular biology and biotechnology training: a practical and alternative approach. Creat Educ 2015;6(8):762–772. DOI: 10.4236/ce.2015.68079
11. Foresta C, Ferlin A, Gianaroli L, et al. Guidelines for the appropriate use of genetic tests in infertile couples. Eur J Hum Genet 2002;10(5): 303–312. DOI: 10.1038/sj.ejhg.5200805
12. Baird DT, Collins J, Egozcue J, et al. Fertility and ageing. Hum Reprod Update 2005;11(3):261–276. DOI: 10.1093/humupd/dmi006
13. Warburton D, Fraser FC. Spontaneous abortion risks in man: data from reproductive histories collected in a medical genetics unit. Am J Hum Genet 1964;16(1):1–25. PMID: 14131871.
14. Vicdan A, Vicdan K, Günalp S, et al. Genetic aspects of human male infertility: the frequency of chromosomal abnormalities and Y chromosome microdeletions in severe male factor infertility. Eur J Obstet Gynecol Reprod Biol 2004;117(1):49–54. DOI: 10.1016/j.ejogrb.2003.07.006
15. Turan GA, Kaya I, Genç M, et al. Chromosomal abnormalities and polymorphisms among couples with recurrent in vitro fertilization (IVF) failure. Sifa Med J 2015;2(3):49. DOI: 10.4103/2148-7731.166854
16. Zhang HG, Wang RX, Li LL, et al. Male carriers of balanced reciprocal translocations in Northeast China: sperm count,

reproductive performance, and genetic counseling. Genet Mol Res 2015;14(4):18792–18798. DOI: 10.4238/2015
17. Verma RS. Heterochromatin: Molecular and Structural Aspects. Cambridge University Press; 1988.
18. Ferlin A, Arredi B, Speltra E, et al. Molecular and clinical characterization of Y chromosome microdeletions in infertile men: a 10-year experience in Italy. J Clin Endocrinol Metab 2007;92(3):762–770. DOI: 10.1210/jc.2006-1981
19. Krausz C, Forti G, McElreavey K. The Y chromosome and male fertility and infertility 1. Int J androl 2003;26(2):70–75. DOI: 10.1046/j.1365-2605.2003.00402.x
20. Hellani A, Al-Hassan S, Iqbal MA, et al. Y chromosome microdeletions in infertile men with idiopathic oligo-or azoospermia. J Exp Clin Assist Reprod 2006;3(1):1–6. DOI: 10.1186/1743-1050-3-1
21. Kamp C, Hirschmann P, Voss H, et al. Two long homologous retroviral sequence blocks in proximal Yq11 cause AZFa microdeletions as a result of intrachromosomal recombination events. Hum Mol Genet 2000;9(17):2563–2572. DOI: 10.1093/hmg/9.17.2563
22. Girardi SK, Mielnik A, Schlegel PN. Submicroscopic deletions in the Y chromosome of infertile men. Hum Reprod 1997;12(8):1635–1641. DOI: 10.1093/humrep/12.8.1635
23. Krausz C, Quintana-Murci L, McElreavey K. Prognostic value of Y deletion analysis: what is the clinical prognostic value of Y chromosome microdeletion analysis? Hum Reprod 2000 ;15(7): 1431–1434. DOI: 10.1093/humrep/15.7.1431
24. Ferlin A, Moro E, Rossi A, et al. The human Y chromosome's azoospermia factor b (AZFb) region: sequence, structure, and deletion analysis in infertile men. J Med Genet 2003;40(1):18–24. DOI: 10.1136/jmg.40.1.18
25. Kuroda-Kawaguchi T, Skaletsky H, Brown LG, et al. The AZFc region of the Y chromosome features massive palindromes and uniform recurrent deletions in infertile men. Nat Genet 2001;29(3):279–286. DOI: 10.1038/ng757
26. Hopps CV, Mielnik A, Goldstein M, et al. Detection of sperm in men with Y chromosome microdeletions of the AZFa, AZFb and AZFc regions. Hum Reprod 2003;18(8):1660–1665. DOI: 10.1093/humrep/deg348
27. Mafra FA, Christofolini DM, Bianco B, et al. Chromosomal and molecular abnormalities in a group of Brazilian infertile men with severe oligozoospermia or non-obstructive azoospermia attending an infertility service. Int Braz J Urol 2011;37(2):244–250. DOI: 10.1590/s1677-55382011000200011