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Original Article

Microdeletions in the Y chromosome in cases of male infertility in a population of West Bengal

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ABSTRACT

Introduction: Infertility is a burning problem in gynecological, andrological, endocrine and genetic practice. Of the myriad factors responsible for male infertility, which may be manifested as oligozoospermia or azoospermia, the exact causes of the latter are still unknown or debatable. Among the known parameters, the occurrence of microdeletions in the long arm of the Y chromosome are of great importance, as they have been consistently associated with defects in spermatogenesis. The microdeletions of the Y chromosome have been mapped to three regions in interval 6 named azoospermia factor regions (AZF), AZFa, AZFb and AZFc.

Methods: In the present study 80 males suffering from oligozoospermia or azoospermia were taken from both rural and urban infertility clinics and subjected to Polymerase Chain Reaction (PCR) of DNA from blood samples using a total of 11 STS primers. These primers correspond to different segments of the AZF regions (AZFa, AZFb and AZFc) and are known as Sequence Tagged Sites (STS). This was followed by agar gel electrophoresis to look for deletions in the AZF regions corresponding to the STF primers.

Result: These tests were able to detect microdeletions in the long arm of the Y chromosome in 4 patients.

Discussion: In majority of patients PCR detects no abnormality but in cases having microdeletions, appropriate advice could be given to the patients. These patients were told to avoid the use of their sperm in assisted reproduction procedures and accept the use of donor sperm or adoption procedures as a solution to their problems of infertility.

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1. Introduction

Infertility is a major health problem affecting 10–15% of couples of reproductive age group seeking to have children. Primary infertility refers to the couples who have never achieved a pregnancy despite 12 months of unprotected intercourse. Available data indicate that in approximately 50% cases, a male factor is responsible for primary infertility. A significant proportion of infertile males show oligozoospermia or azoospermia. Such alteration in sperm production may be due to some known causes e.g. trauma or torsion to the testes, bilateral cryptorchidism, endocrine disorders, infections etc. However the cause of male infertility is unknown in upto 50% cases and recently researchers are focusing on an important genetic factor in the form of Y chromosome microdeletion which has a positive correlation with primary male infertility.

The Y chromosome is not essential for life. Sex determination has long been regarded as the only function related to the Y chromosome. Tiepolo and Zuffardi, in 2006[1] were the first to hypothesize a positive correlation between Y chromosome deletion and male infertility. They observed large deletions including the entire heterochromatic regions (Yq12) and an undefined amount of the adjacent euchromatic part (Yq11) in 6 sterile males with azoospemia by examining the karyotype of 1170 men. They postulated that a genetic factor located in Yq11 was important for male spermatogenesis. These genes or gene cluster was defined as "azoospermia factors" (AZF). Further studies by Reijo et al published in Lancet 1996[2]; Pryor et al published in N. Eng. J. Med 1997[3] extensively proved that a specific gene or gene cluster within interval 6 on the Y chromosome is missing in 7–13% of azoospermic and oligozoospermic male patients with infertility. Vollrath et al[4] in their study proved that deletion occurs in interval 5–7, a region band in Yq11.23 and there were 3 regions in the deletion interval i.e. AZFa, AZFb, AZFc (Fig. 1). The candidate genes which express AZFb and AZFc encode testis specific RNA binding proteins, but the function of those genes expressing AZFa are not clearly known. In India, Dr. K. Thangaraj, Dr. B.N. Chakraborty, and their coworkers in 2003[5] made a combined study in Hyderabad and Kolkata. They analyzed 340 azoospemic men and found that 8.5% showed Y chromosome deletion. Mitra and his coworkers in 2008[6] observed Y chromosome microdeletions in the AZFa, AZFb and AZFc regions by PCR in 5.29% of infertile males all of whom were azoospermic. In another study by Dr. Athalye et al,[7] reveals 8.9% cases of Y chromosome deletion by using multiplex P.C.R. technique.

To summarize, it is now established that male infertility due to oligospermia and azoospermia is frequently associated with microdeletions in the long arm of the Y chromosome. The genes collectively implicated in these microdeletions are called azoospermia factors (AZF). These AZFs occur in the regions Yq11.21, Yq11.22 and Yq11.23 known as AZFa, AZFb and AZFc. A multitude of Sequence Tagged Sites (STS) have been generated and mapped for the entire Y chromosome. These STS are known sequences of genomic DNA from the AZF regions that can be amplified by PCR using suitable STS primers. 300 sequence tagged sites (STS) have been generated and mapped for the above three AZF regions. Microdeletions in the Y chromosome are looked for in these Sequence Tagged Sites.

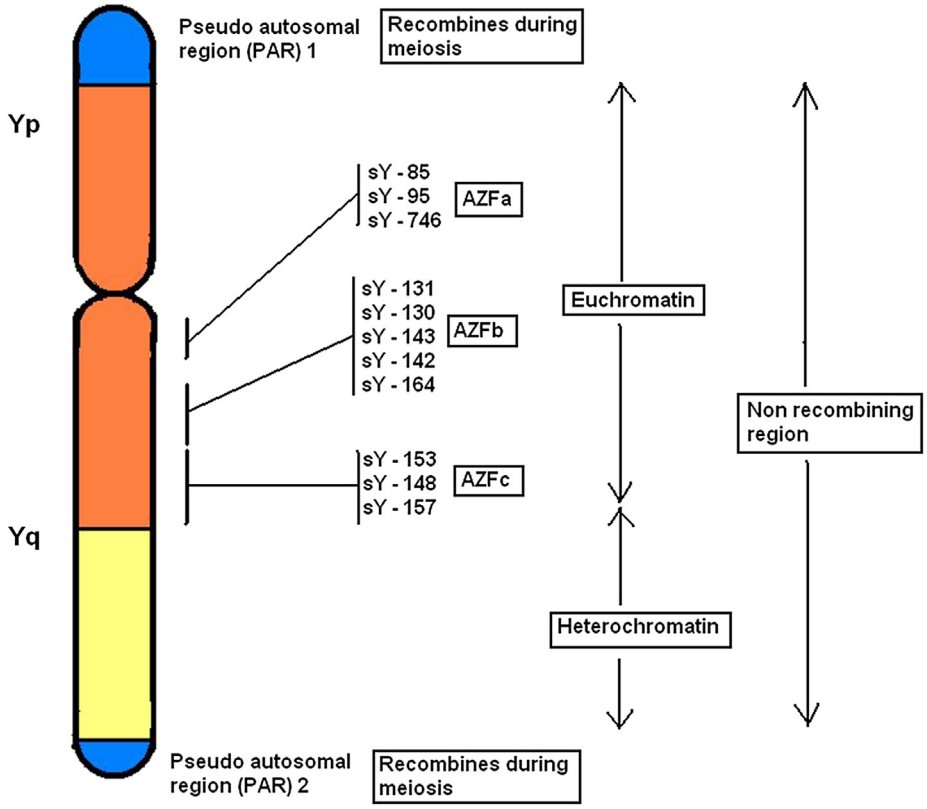

Fig. 1 – The human Y chromosome.

In our study an attempt has been made to find out correlation between Y chromosome microdeletion and etiology of male infertility in Eastern India.

2. Materials and methods

The main objective of this study was to probe the role of any genetic factor in the population of infertile males with azoospermia or serve oligozoospermia and a comparison between urban and rural population so that this study may help in effective evaluation, counseling, rehabilitation and assist the physicians to take the proper decision for assisted reproduction or adoption.

Male partners of the affected couples with primary infertility attending the outpatient department of the following hospitals were chosen to participate in the study with full informed consent of both partners.

- Nil Ratan Sircar Medical College & Hospital, Kolkata.
- Dr. B.C. Das Memorial Medical Complex & Research Centre, Tarakeswar, Hooghly.
- Bankura Sammilani Medical College, District Bankura, West Bengal.

In all patients, a proper history was taken and some routine investigations were done which included routine blood count, hormonal assays and semen analysis.

2.1. *Selection criteria*

- Age group 25–45 years.
- 80 males with oligospermia or azoospermia were selected for the study.
- Either azoospermic (no sperm in semen) or severe oligozoospermic (<1 million sperm/ml of semen)
- No history of medical illness like mumps, hypothyroidism testicular feminization syndrome or any surgical disease or intervention like trauma or torsion to the testis, orchidopexy or any obstruction to their seminal passage.

2.2. *The following investigations were done on the selected patients*

1) Karyotyping from blood lymphocytes and giemsa trypsin banding to note any numerical or structural abnormalities of the chromosome.
2) Polymerase chain reaction of D.N.A. of blood sample by using P.C.R. primers sY-85, sY-95 & sY-746 from AZFa region, sY-130, sY-131, sY-142, sY-143 & sY-164 from AZFb region, sY-148, sY-153 & sY-157 from AZFc region of the Y chromosome, in P.C.R. machine (Bio-Rad, 24 well thermocycler), to detect base pair sequences of Y chromosome and identify microdeletions if any.

To check whether the PCR generated the anticipated DNA fragments, agarose gel electrophoresis was employed for size separation of the PCR products. The size(s) of PCR products was determined by comparison with a DNA ladder (a molecular weight marker), which contained DNA fragments of known size, run on the gel alongside the PCR products (Fig. 2).

3. Results

The results of the present study are presented in a tabular form in Table 1.

The analysis of the data reveals that in urban population out of 31 oligozoospemic patients, 1 patient showed Y chromosome microdeletion (sY-142 of AZFb region) and out of 9 azoospemic patients, 1 patient had Y chromosome microdeletion (sY-164 of AZFb region), (Fig. 2). So out of 40 male subjects, 5% had Y chromosome microdeletion.

Similarly in rural population, out of 30 persons suffering from severe oligozoospermia, no patient shows Y chromosome microdeletions. On the other hand, out of 10 male subjects having azoospermia, 2 persons were found to have Y chromosome microdeletion (sY-164 & sY-130 of AZFb region). This corresponds to 20% in azoospemic sample and 5% in total rural sample. To summarize, out of 61 oligozoospemic males, 1 (1.64%) and out of 19 azoospemic males, 3 (15.79%) show Y chromosome microdeletion. In the total sample ($n = 80$) Y chromosome deletions were found in 5% cases and there was no significant urban-rural variation.

In most subjects, the karyotype was essentially normal and revealed no anomalies.

4. Discussion

The determination of segments of the Y chromosome controlling spermatogenesis (azoospermia factors – AZF) and the

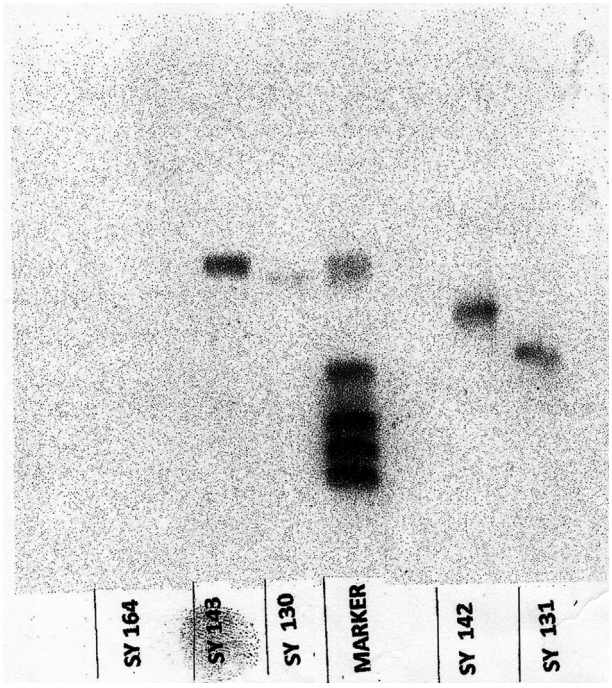

Fig. 2 – PCR analysis of Y chromosome with agarose gel electrophoresis showing microdeletion in the AZFb region at sY-164.

Table 1 – Distribution of cases of Y chromosome microdeletion in the present study.

	Type of semen	Y chromosome microdeletion	Other genetic abnormalities	No genetic causes
Urban ($n = 40$)	Oligospermia ($n = 31$)	01	00	30
	Azoospermia ($n = 09$)	01	00	08
Rural ($n = 40$)	Oligospermia ($n = 30$)	00	00	30
	Azoospermia ($n = 10$)	02	00	08

ease of location of their presence or deletion of such segments by PCR, using sequence tagged sites (STS) as markers, has resulted in a deluge of information from different sources. The European Academy of Andrology (EAA) recommends testing with 6 STS markers, 2 each from the AZFa, AZFb and AZFc regions of the Y chromosome. Previous studies in India have shown that this selection detects fewer microdeletions. Therefore, the markers prescribed by EAA alone are not suitable for the diagnosis of Y chromosome microdeletions in infertile males (Sachdeva et al, 2008).[8] The greatest numbers of microdeletions are commonly present in the AZFb region (Vogt et al, 1996).[9] Some studies have on the other hand, stressed the importance of the DAZ gene on the AZFc region as an important azoospermic factor (Liow et al, 1998).[10] However, it has been found that these studies are more relevant if they are carried out using markers that are commonly found to be deleted in the Y chromosomes of the local population. Such markers are known as ethnic or indigenous markers. Ideally, in looking for Y chromosome microdeletions, STS segments corresponding to AZFa, AZFb and AZFc regions should be used, for the most comprehensive coverage. In the present study, all of the STS markers used have been taken from the AZFa, AZFb & AZFc regions. The selection of individual markers was done by considerations of local prevalence as well as commercial availability of such STS markers for PCR. However, all of the microdeletions detected were from the AZFb region. Other studies have also shown a much greater prevalence of microdeletions in the AZFb loci (Elhawary et al, 2002).[11] Oliva et al (1998)[12] reviewed the published series of azoospermia and demonstrated that the worldwide frequency of microdeletions was 13%. Among infertile men, the prevalance of Y microdeletions is approximately 7% (1–35%).

The occurrence of microdeletions is greater in cases of azoospermia (3 out of 19 cases, 15.8%), than in oligospermia (01 out of 61 cases, 1.6%), as evidenced in our study. Similar results were obtained by Elhawary et al,[11] and Ceylan et al (2009).[13]

Our study reveals that 1.63% oligozoospemic males, and 15% of azoospemic males show Y chromosome microdeletion. In reality, the majority of microdeletions have been found in men with azoospermia and severe oligospermia, because in most of the studies published so far, the analyses were limited to patients with severe defects of spermatogenesis.

In the present study, out of the total sample of infertile males ($n = 80$), Y chromosome deletions were found in 5% cases. This tallies with the results of larger studies like those of Mitra et al,[6] in which Y chromosome microdeletions were observed in 9 out of 170 (5.29%) infertile males.

In the present study, environment and lifestyle seemed to have no effect on sperm count as evidenced by the similar results obtained in both rural and urban populations.

However, from Table 1, it is evident that no causes for oligospermia/azoospermia were detected in 86 out of 90 cases (95.5%). This shows us that we are still a long way towards understanding the factors influencing spermatogenesis, both qualitatively and quantitatively. Perhaps a few other cases of genetic anomalies would be evident after more extensive and sophisticated tests.

In this context, it should be borne in mind that the majority of the Y chromosome deletions arise de novo. In some cases, the deletion may be transmitted from a fertile father to an infertile son (Rolf et al, 2002).[14] The origin of the microdeletions is not clearly understood. Microdeletions may arise in the testes, in fertilized eggs and embryos; microdeletions prevent the formation of spermatogonia in the fetus, resulting in impaired spermatogenesis in the adult. The high frequency of Y microdeletions suggests that the Y chromosome is susceptible to spontaneous loss of genetic material (Zamani et al, 2006).[15]

As all the subjects of the present study had a normal karyotype, this study emphasizes the need of PCR based studies to elucidate microdeletions in the Y chromosome (Omrani et al, 2006).[16]

5. Conclusion

Karyotyping and Y chromosome study should be an important second line investigation study to determine the cause of male infertility in men with azoospermia or oligozoospermia. Detection of microdeletions avoids unnecessary hormonal and other unproven treatments in a vain attempt to boost the sperm count (Rajneesh et al, 2010).[17] This also helps to predict the effectiveness of assisted reproductive technologies in men with specific Y chromosome microdeletion. In some parts of the world, detection of microdeletions in the AZF regions is done as a postnatal screening test for male infants. This however, is not common in India. Screening tests will also determine the proportion of males in a population with Y chromosome microdeletions and the incidence of infertility in them after marriage.

A case may also be made out in advising infertile couples to choose a female child in case of Y chromosome deletions in opting for assisted reproduction methods (Abilash et al, 2010).[18]

Conflicts of interest

All authors have none to declare.

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