INDIAN COUNCIL OF MEDICAL RESEARCH | NATIONAL INSTITUTE FOR RESEARCH IN REPRODUCTIVE HEALTH

POLICY BRIEF

FEBRUARY 2020

Y Chromosome Microdeletion Testing for Male Infertility

Who is this policy brief for ?

- Reproductive Biology Maternal Health and Child Health (RBMH & CH) Division, Indian Council of Medical Research
- Healthcare policy makers
- Assisted Reproductive Technology (ART) clinics in India
- Urologists, Andrologists, Gynecologists and ART specialists including Embryologists involved in infertility management in public and private hospitals in India
- Public and private diagnostic laboratories offering genetic testing and counselling in India

Dr. Deepak Modi and Dr. Stacy Colaco

Molecular and Cellular Biology Laboratory,
ICMR- National Institute for Research in Reproductive Health,
JM Street, Parel, Mumbai 400 012

EXECUTIVE SUMMARY

Male infertility occurs due to lack of sperm production (azoospermia) or low sperm production (oligozoospermia), and microdeletions of the Y chromosome account for infertility in 8-10% of men. At present, in India, no guidelines exist regarding the testing of Y chromosome microdeletions. In this policy brief, we recommend that it should be mandatory to test infertile males for Y chromosome microdeletions. The assays that use specific markers for the Indian population must only be used for testing. If Y chromosome microdeletions are identified, assisted reproduction should not be offered to such couples. If the couple yet desires assisted reproduction, they must be informed that all their sons will carry the deletion and hence perpetuate male infertility in their future generations.

BACKGROUND

National Health Portal of India estimates that nearly 1 in every 6 couples is estimated to be infertile in the country [1]. Of these, 40% are due to male factor infertility like azoospermia (no sperm in semen) or oligozoospermia (low sperm count and motility in semen). Amongst the various causes of azoospermia and oligozoospermia, nearly 8% of infertile males carry Y chromosome microdeletions [2,3] .There are three types of Y chromosome microdeletions, namely AZFa, AZFb, and AZFc. In addition, the gr/gr sub deletion (or partial deletion) also poses an increased risk of male infertility [2,4] .

METHODOLOGY

Several research labs in India including ICMR-NIRRH have conducted studies on Y chromosome microdeletions. We collected the published data from different parts of the country on Y chromosome microdeletions in men with azoospermia and oligozoospermia. We collected data on commonly used markers for detection of Y chromosome microdeletions in India. We also collected Indian and global data on outcomes of fertility treatments and other health risks in men who have Y chromosome microdeletions.

RESULTS AND CONCLUSIONS

The frequency of Y chromosome microdeletions in infertile Indian men is ~8% with high variations in different states (Figure 1). We observed that most research labs use only the 6 genetic markers recommended by

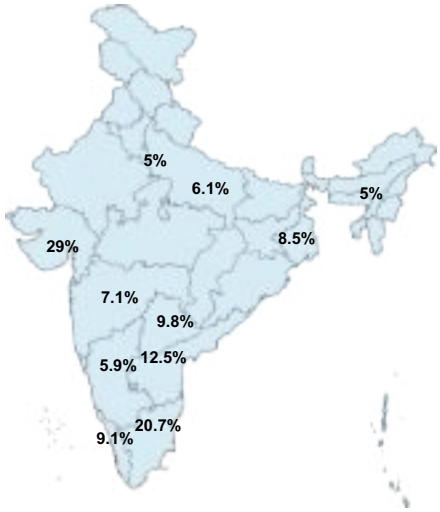

Figure 1: Data on frequency of Y chromosome microdeletions in Indian infertile men

European Academy of Andrology and the European Molecular Genetics Quality Network [3]. We found that the 6 markers are not sufficient to detect Y chromosome microdeletions in Indian men, as they miss out nearly 50% of cases [3]. Thus, Y chromosome microdeletions may be underdiagnosed in many Indian labs.

To address this problem, we identified 16 genetic markers that will accurately diagnose Y chromosome microdeletions and developed a method that makes use of these markers simultaneously to detect the AZFa, AZFb and AZFc deletions (Figure 2). Absence of any one of the bands in the assay would indicate a Y chromosome microdeletion. This assay is ideal for screening of infertile males choosing to undergo Assisted Reproductive Technologies (ART) to achieve parenthood. Based on the results of this assay one can determine if the azoospermia or oligozospermia is due to Y chromosome microdeletion. Thus, the result of this test will help the doctors to take a decision if one should undergo Testicular Sperm Extraction (TESA) - Intracytoplasmic Sperm Injection (ICSI) for biological parenthood. The test will also help to determine if the embryos obtained after TESA-ICSI will be healthy and would result in successful pregnancy.

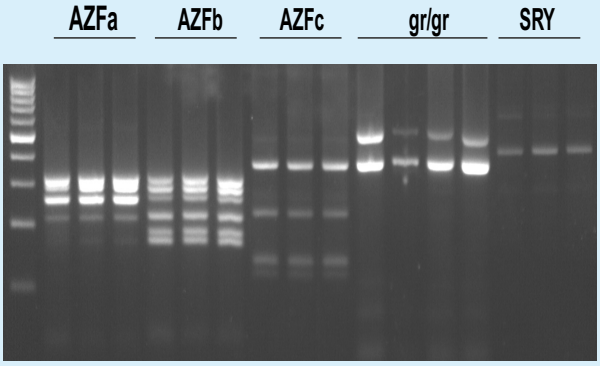

Figure 2:
Test for determining Y chromosome microdeletions using multiplex PCR. The lanes labeled as AZFa, AZFb, AZFc and gr/gr show bands for each specific marker. The absence of one or more bands in AZFa, AZFb, AZFc or gr/gr would mean a microdeletion. The lane labeled as SRY is positive control and must be present in all male DNA samples

The consequences of Y chromosome microdeletions

Many infertile men are prescribed medical treatments that are known to improve sperm count and motility. However these therapies will not work if there is a Y chromosome microdeletion and thus the patient should not be subjected to unnecessary treatment regimens. For those couples desiring biological parenthood, TESA followed by ICSI is the method of choice offered to men with azoospermia and oligozoospermia. However, if there are AZFa and AZFb deletions, there is a very small chance of retrieving sperm after TESA[2,5]. Although sperm can be retrieved by TESA in men with AZFc or gr/gr partial deletions, the resulting embryos after ICSI have low chance of fertilization; the embryos are of poor quality and often will have genetic defects [2,4,7]. Thus, TESA - ICSI will not be successful in most men with Y chromosome microdeletions.

All males inherit the Y chromosome exclusively from their fathers. Thus, in men with Y chromosome microdeletions, the deletion will be transmitted from fathers to all their sons born after TESA-ICSI[2]. In many cases the deletions also expand in the next generation and the defect becomes more severe [2]. This will perpetuate infertility in the future generations (Figure 3). Thus, the couples need to take an informed decision prior to accepting any assisted reproductive technologies for biological parenthood.

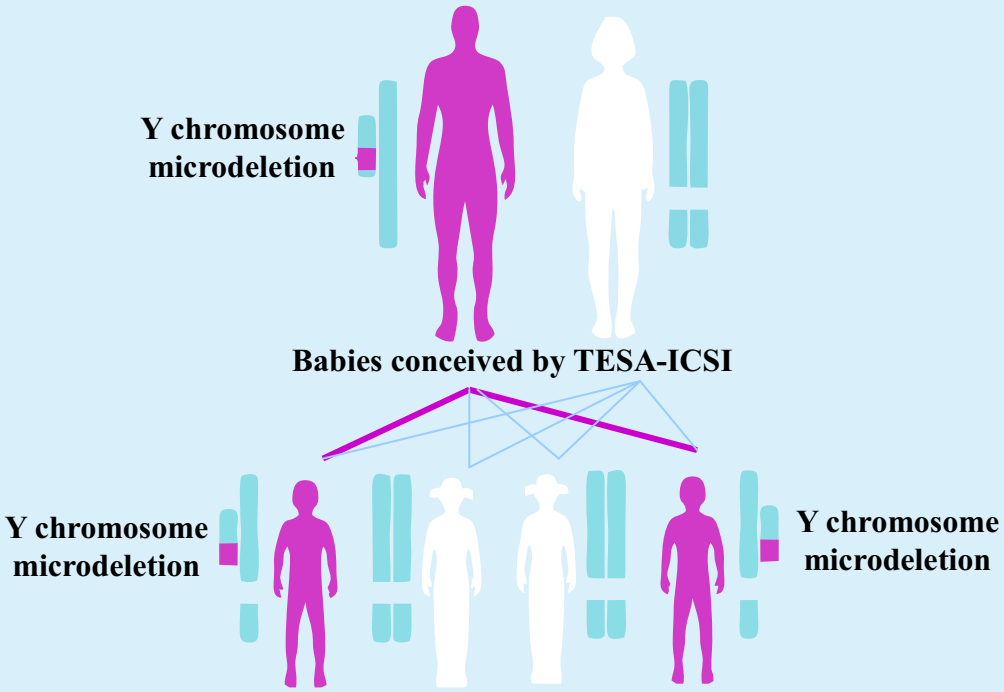

Figure 3: Infertile men undergo Testicular Sperm Aspiration (TESA) followed by Intracytoplasmic Sperm Injection (ICSI). Of the babies thus born, the Y chromosome microdeletion will be transmitted to all sons from fathers making them also infertile.

In addition to infertility, low fertilization rates and poor embryo quality, research has also shown that the men with Y chromosome microdeletions have increased risk of neuropsychiatry, Cardio Vascular Disease (CVD) and cancers as they age [2,6].

Thus, men and their sons with azoospermia and oligozoospermia who have Y chromosome microdeletions are also at high health risks along with infertility (Figure 4).

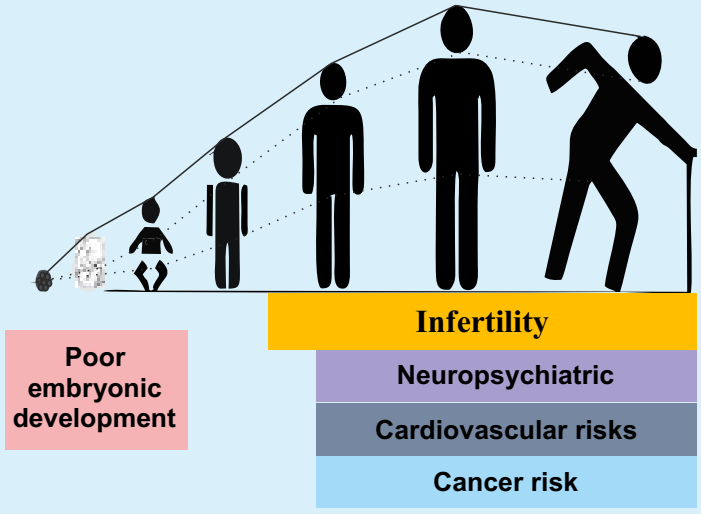

Figure 4: Health risks due to transmission of Y chromosome microdeletions

RECOMMENDATIONS

Despite the alarming increase in numbers of infertile men in India, no guidelines exist regarding their genetic testing. As a result, men with Y chromosome microdeletions continue to suffer from related health conditions. Further, infertile men are offered ART without testing and sufficient counselling thus putting the future generation at increased risk of infertility and other adverse health outcomes. This policy brief makes the following recommendations regarding Y chromosome microdeletion testing in India.

1) The National Guidelines for Accreditation, Supervision & Regulation of ART Clinics in India must make Y chromosome microdeletion testing mandatory for men with azoospermia and oligozoospermia.
2) Y chromosome microdeletions testing must be done with assays that use specific markers for the Indian population, and genetic testing laboratories must ensure that this is accurately adhered to.
3) If Y chromosome microdeletions are identified, the men should not be subjected to any treatment for enhancing sperm count/motility as they will not be benefited from such therapies.
4) If AZFa or AZFb deletions are found, do not offer TESA, as the chance of finding sperm is extremely low. TESA may be attempted if AZFc or partial deletions are reported.
5) However, active offering of TESA-ICSI to men with any type of Y chromosome microdeletions must be avoided, as embryo quality and chance of pregnancy are poor .
6) All men with Y chromosome microdeletions must be informed of the other adverse health risks they may face in future and recommend follow-up with other specialists for better management.
7) If a man with Y chromosome microdeletion and his partner yet desire ART, they must be informed that all their sons but not daughters will carry the deletion and hence male infertility will be perpetuated in their future generations. Additionally, they must be counseled that the males in their future generations will also have higher adverse health risks.
8) There is a need to develop uniform guidelines for genetic evaluation and management of male infertility.
9) There is a necessity to develop curriculum in “androgenetics” for Urologists, Obstetricians, Gynecologists, IVF specialists and Geneticists for better health care of infertile men in India.
10) Academic societies like Indian Society of Assisted Reproduction (ISAR), Indian Fertility Society (IFS), Indian Society for Studies in Reproduction and Fertility (ISSRF), Indian Society of Human Genetics (ISHG) must widely disseminate this policy to the stakeholders in the country.

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REFERENCES

1. National health portal of India https://www.nhp.gov.in/disease/reproductive-system/infertility
2. Colaco S, Modi D. Genetics of the human Y chromosome and its association with male infertility. Reprod Biol Endocrinol. 2018;16:14.
3. Sen S, Pasi AR, Dada R, Shamsi MB, Modi D. Y chromosome microdeletions in infertile men: prevalence, phenotypes and screening markers for the Indian population. J Assist Reprod Genet. 2013;30:413–22.
4. Sen S, Ambulkar P, Hinduja I, Zaveri K, Gokral J, Pal A, Modi D. Susceptibility of gr/gr rearrangements to azoospermia or oligozoospermia is dependent on DAZ and CDY1 gene copy deletions. J Assist Reprod Genet. 2015;32:1333–1341.
5. Mascarenhas M, Thomas S, Kamath MS, Ramalingam R, Kongari AM, Yuvarani S, Srivastava VM, George K. Prevalence of chromosomal abnormalities and Y chromosome microdeletion among men with severe semen abnormalities and its correlation with successful sperm retrieval. J Hum Reprod Sci. 2016; 9:187-193.
6. Colaco S, Modi D. Consequences of Y chromosome microdeletions beyond male infertility. J Assist Reprod Genet. 2019;18:1-9.
7. Colaco S, Sakkas D. Paternal factors contributing to embryo quality. J Assist Reprod Genet 2018;35:1953-68.