Genetic Study in Infertile Male Patients with Asthenozoospermia (AZS)

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Abstrak – Purposes: Abnormally low percentage of progressive motile sperms in semen leads to Asthenozoospermia, mainly found in infertile male patients. Although the success rate of Assisted Reproductive Techniques (ARTs) in AZS is better, chromosomal abnormalities in such patients can lead to repeated failures of ARTs. Dermatoglyphics plays role of a diagnostic tool in screening genetically transmitted diseases. So to reduce financial and psychological stress of failure in treatment of infertile patients. study was conducted by doing Karyotyping as well as dermatoglyphics of such infertile male patients with AZS.

Method: Karyotypes of 17 infertile AZS male patients along with control were studied using standard methods. Dermatoglyphics were obtained using roller ink method, and statistically analyzed.

Results: Very high incidence i.e. 70.29 % (12/ 17) of chromosomal aberrations was found in present study mainly in patients with repeated failures in ARTs. Microdeletion on chromosome no. 2 was significant finding along with other numerical as well as structural abnormalities. Dermatoglyphics also revealed statistically significant findings.

Conclusion: Chromosomal study in case of infertile male patients with asthenozoospermia proceeding for ARTs can help the treating doctors to decide about the line of treatment and the patients to avoid financial and psychological stress of failure in treatment.

Key Words – Asthenozoospermia, chromosomal abnormalities, Dermatoglyphics, failures of ARTs, infertility, Microdeletion.

1 Introduction

The incidence of Asthenozoospermia as a cause of infertility is 38% as recently quoted by L. L. Penney 8 who studied 587 infertile patients. Asthenozoospermia (AZS) is a condition in which percentage of progressive motile sperm is abnormally low. In men it is defined as < 25% rapid motility or < 50% progressive motility in a semen sample (WHO, 1992)12. It decreases the sperm quality and is therefore one of the major causes of infertility or reduced fertility in men. A number of studies have
been conducted to study chromosomal abnormalities in Azoospermic and Oligozoospermic infertile male patients but very few have focused on Asthenozoospermia (AZS). AZS may exist as an isolated disorder, in combination with other sperm anomalies or as a part of syndromic association.

Till date only a few genes constituting the cilia or flagella structure have been associated with isolated AZS in humans whereas several other genes are known to be involved in syndromic form of AZS including Primary Ciliary Dyskinesia (PCD) and Kartagener syndrome (KS) quoted by Zuccarello D. (July 2008)13.

The success rate of Assisted Reproductive Techniques (ARTs) like Intra Uterine Insemination (IUI) and Intra Cytoplasmic Sperm Injection (ICSI) in infertile male patients with AZS is better than azoospermia. But in case of failures of ARTs in AZS male patients, we can think of chromosomal abnormalities being a reason for it.

It has been also observed that chromosomal abnormalities have been transmitted to fetuses that are the outcome of ARTs. So it becomes essential to focus our study on genetic study of infertile male patients with AZS to avoid the transmission of abnormal genetic constitution to next generation as well as to understand the probable reason behind the failures of ARTs in them.

The role of Dermatoglyphics as a diagnostic tool in screening genetically transmitted diseases is becoming increasingly popular because

1. Ridge configuration is genetically determined.
2. Chromosomal aberrations alter the ridges.
3. Ridges are formed in the 18th week of IUL and remain the same throughout life.

So this pilot study was undertaken to analyze incidence of chromosomal aberrations in infertile male patients with asthenozoospermia.

Aims and objectives:

1. To study the chromosomal and dermatoglyphic patterns of infertile male patients with asthenozoospermia.
2. To try and co-relate dermatoglyphics with chromosomal aberrations in AZS.
3. To try and co-relate chromosomal aberrations and repeated failures after ARTs in patients with AZS which will help to decide about the line of treatment & genetic counseling.
4. To look for a marker for mass screening in male infertility.

2 Material and methods

In present study, 17 infertile male patients coming to infertility clinic for ART with clinically diagnosed AZS according to WHO criteria were selected along with 17 fertile healthy males as control.

Chromosomal study was pursued by collecting 2 ml of blood in heparinized syringe under aseptic conditions after taking the written consent of the patient and incubating blood sample with culture medium for 68 hours in BOD incubator and also processed further in genetic laboratory, Dr. D.Y. Patil Medical College, Pune. The chromosomes were stained with Giemsa banding technique10. Chromosomes were identified and arranged in groups A to G so also X and Y chromosomes as per their characters and the configurations of light and dark bands over them. Karyotypes were analyzed.
for chromosomal abnormalities. Dermatoglyphic study was undertaken by using roller ink method with Kores duplicating ink. Prints of both palms and soles were taken. Patterns were analyzed using Henry’s system as

- Arches (simple(F1) and tented(F2)),
- Loops (double(F4), ulnar(F5), radial(F6), central pocket(F7)),
- Whorls (spiral(F9), concentric(F10), elliptical(F11), central pocket(F13),)
- Composite(F8, F12) and
- Vestige pattern (F3) for both palmar and plantar dermatoglyphics.

Dermatoglyphic Patterns:

- F1. Simple Arch
- F2. Tented Arch
- F3. Vestige Pattern
- F4. Double loop Whorl ('S' shaped whorl)
- F5. Radial Loop
- F6. Ulnar Loop
- F7. Central pocket Loop
The palmar and plantar dermatoglyphic patterns so also the karyotypes were compared with that of controls to know if they are differing from the normal patterns and were statistically analyzed.

3 Observations

In present study,

3.1 Chromosomal study revealed:

Incidence of chromosomal aberrations in infertile male patients with AZS is 70.59% (12/17 cases).

4 (23.5%) patients’ karyotypes showed more than one chromosomal aberrations. Further distribution of different chromosomal abnormalities is as follows:
Table No. 1 Distribution of different chromosomal abnormalities

<table>
<thead>
<tr>
<th>No</th>
<th>Microdeletion</th>
<th>Homozygous</th>
<th>Heterozygous</th>
<th>Photograph No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.</td>
<td>del(3)(p26)</td>
<td>Homo 5.9%(1/17)</td>
<td>Hetero 11.7%(2/17)</td>
<td>2</td>
</tr>
<tr>
<td>3.</td>
<td>del(13)(q13)</td>
<td>Homo 5.9%(1/17)</td>
<td>Hetero 5.9%(1/17)</td>
<td>3</td>
</tr>
<tr>
<td>4.</td>
<td>del(22)(q12)</td>
<td>Homo 5.9%(1/17)</td>
<td>Hetero 5.9%(1/17)</td>
<td>4</td>
</tr>
<tr>
<td>5.</td>
<td>del(21)(P11.1ter)</td>
<td>Homo 17.6%(3/17)</td>
<td>Hetero 5.9%(1/17)</td>
<td>4</td>
</tr>
</tbody>
</table>

3.1.1 Ideogram

Chromosome No.2 & 3

Chromosome No. 9
3.1.2 Photographs of Few Interesting Karyotypes

Karyotype Photograph no. 1:

Karyotype Photograph no. 2:

Findings:
- 46,XY; del(2)(p24)hetero, pericentric del(9)(p12→q13)hetero, and pericentric translocation on homologous chromosome 9
- del(22)(q12)hetero.
Karyotype Photograph no. 3:

Karyotype Findings: - 46,XY del(3)(p26)hetero.
Karyotype Photograph no. 4:

Findings: 46,XY del(21)(p11.1→ter)(q12→ter)homo.

3.2 Dermatoglyphics:

Dermatoglyphic study revealed:

Findings which are statistically significant are,
Planter dermatoglyphics showed:
- Presence of double loop whorl in toe tip pattern (Photograph no. 5).
- Presence of elliptical whorl in ball region of both right as well as left foot (Photograph no. 6).
Planter Dermatoglyphics

Photograph no. 5:
Double loop whorl in toe tip pattern seen in 2nd - 4th toes.

Photograph no. 6:
Elliptical whorl in ball region
4 Discussion:

Spermatogenesis is one of the most complex cell differentiation processes known, involving about 2,300 genes in the regulation of testicular development, germ cell development and maturation. Although investigators estimate that almost 50% of patients with idiopathic male infertility have a genetic contribution, most of these genetic factors remain to be elucidated.

Ann C Chandley et al (1975) observed that genetic or chromosomal factor exerts its effects on gamete formation and function. Hence chromosomal analysis is widely recommended for male partner undergoing ART by many authors like De Brackeleer and Doet (1991), Nagvenkar P et al (2005) etc.

Myriam Ghorbel et al (2012) studied infertile men with poor semen quality which revealed incidence of chromosomal abnormality being 13.5% in severe oligo-asthenozoospermic.

Plaseska-KD (2012) mentioned, the most common genetic causes of male infertility observed are sex chromosomal aneuploidies like 47XXY, 47XYY and 46XXmales so also Y chromosome microdeletions mainly AZF regions.

Li Fu, et al (2012) stated the incidence of autosomal abnormalities being 26.62% of abnormal karyotypes and interestingly out of that 65.85% had abnormal karyotype involving chromosome 1 or 14, suggesting that some genes present on these chromosomes might play an important role in spermatogenesis.

Alexander N. Yatsenko et al (2010) observed different structural autosomal rearrangements in the 29% (16/55) infertile patients in which they noted balanced translocations, pericentric inversions of chromosomes 1, 2, 4, 7, 9 and paracentric inversion of chromosome 1.

Liesbeth Visser, et al (2011) studied 30 men with isolated asthenozoospermia and identified 10 heterozygous asthenozoospermia-specific mutations in ADYC10, AKAP4, CATSPER1, CATSPER2, CATSPER3, CATSPER4, and PLA2G6. They concluded that given their putative effect on protein structure, their location in conserved sequences or functional domains, and their absence in controls, the identified mutations may be a cause of asthenozoospermia in humans.

In present study 70.29% of infertile male patients with AZS are showing chromosomal aberrations mainly structural in the form of autosomal deletions. Incidence of microdeletions on chromosome no. 2 is significantly high as shown in table 1. Few of these infertile males with AZS which showed chromosomal abnormality, suffered from repeated failures of ARTs

Microdeletion on chromosome no. 2 at P24 with such significant incidence is being reported for the first time in present study in patients with AZS to the best of our knowledge which is also being co-related with dermatoglyphic findings. There might be a gene at this site which is concerned with the motility structure and function of sperm, which may be confirmed by using FISH Technique or PCR.

Modern cytogenetic method allows precise identification of chromosomes and thus helps in studying co-relation between individual chromosomal aberrations and dermatoglyphic features.

Dermatoglyphic study was carried out with the purpose that the chromosomal study is expensive and every patient cannot be subjected for it because of multiple constraints. Dermatoglyphic study is
simple and cost effective tool, can be used for mass screening or at places where the advanced methods of investigations like karyotyping are not available. It was also thought of that findings from dermatoglyphic study can also be used as genetic marker, and patients with positive findings can be further investigated by chromosomal study methods.

Thus the present study shows that there is definite co-relation between chromosomal abnormalities, dermatoglyphics and asthenozoospermia. Incidence of chromosomal abnormalities in present study is very high. Its mainly structural abnormalities like deletions on chromosome no 2 i.e. 2p24, 2p22, 2q12 also on other chromosomes like (3)(p26), (13)(q13), (22)(q12). Apart from these it also revealed terminal deletion on chromosome no 21 (p11.1 ter), (p11.1 ter) and pericentric deletion on chromosome no. 9 (p12 q13) with homologous translocation.

This would be of great help to come to some conclusion about the possibility of cause of infertility, failure of ARTs in spite of best of the treatment in expert hands and repeated abortions in case of ARTs.

May be this is the time to think whether these chromosomal abnormalities in these patients is the cause of failure of ARTs in spite of best of the treatment. If it is so, present study might give a guideline to the treating doctors to decide about the line of treatment and might also help the patients to avoid financial and psychological stress of failure of treatment.

Now we can say that chromosomal study can be an important investigation in case of infertile male patients with asthenozoospermia proceeding for ARTs.

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**Presentation at a meeting:** Received ‘Young Scientist Award’ for Best Poster Presentation on “Genetic study of infertile male patients with asthenozoospermia.” in 3rd International Genetic conference held at Yenepoya University, Mangalore, Karnataka in Feb. 2010