

Research Article

Study of effect of advanced maternal age related risks for down syndrome & other trisomies

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Abstract

Background: Down syndrome is the most frequent live born aneuploidy & recognizable form of mental retardation among all the ethnic groups of human population across the globe. The most common risk factor for DS is advanced maternal age. The aim of this study was to find out risks of advanced maternal age for chromosomal abnormalities.

Materials & Methods: The chromosomal abnormalities were diagnosed by cytogenetic study of 30 clinically diagnosed cases of DS attending paediatric outpatient department (OPD) & admitted in paediatric ward, Civil Hospital, Ahmedabad, as well as cases from B.M. Institute of Mental Health, Aashram Road, Ahmedabad. Their detailed history was taken.

Results: The study revealed that with advanced maternal age the risk of child birth having DS was increased. After age of 35 years the risk of child birth with DS was increased by 43.4% in our study.

Conclusion: As a woman gets older, her risk to have a pregnancy with a chromosome abnormality increases. The most commonly used definition for advanced maternal age is 35 years or more at time of child birth. With advanced maternal age hormonal level, numbers of healthy oocyte for fertilization, telomere length decreases with reduced meiotic recombination. All these factors increase the risk of pregnancy with DS.

Keywords: chromosomal abnormalities, cytogenetic study, down syndrome, karyotype, maternal age, meiotic division

1. Introduction

Ever since the discovery of the extra chromosome was made, various workers have attempted to explore the cause of nondisjunction (NDJ) of chromosome 21. DS can be caused by 3 types of chromosomal abnormalities: free trisomy 21, translocation or mosaicism¹. Important factors in the conception of trisomies are: delayed fertilization, advanced maternal age, increased satellite association; physical, biological & chemical mutagens have also been found to cause NDJ². The present study was performed to ascertain the role of advanced maternal age in the occurrence of trisomy- DS & to detect the origin of the extra chromosome.

Before many years, families were larger & women often continued bearing children until the end of their reproductive age. But now a days with increase in educational level, finding a stable job, securing a higher salary & for increasing career prospects many of couples postpone parenthood. But advanced maternal age is also associated with adverse outcomes in the perinatal period, which may be caused by detrimental effects on decidual & placental

development³.

Free trisomy 21 is characterized by the presence of three complete copies of chromosome 21, generally resulting from NDJ during maternal meiosis & is seen in about 95% of cases. Translocations are attributed to 3-4% of cases, with Robertsonian translocation involving chromosomes 14 & 21 being the most common type. Mosaicism, characterized by some cells containing 46 chromosomes & others with 47 chromosomes, is reported in 1% of DS cases.

Like that of other autosomal aneuploidy, the errors during maternal oogenesis account for about 90% of DS births⁴. Advanced maternal age & altered meiotic recombination are two strong correlates associated with NDJ of chromosome 21 in oocyte⁵.

The paternal age show its adverse effect only when age of the female partner is 35 years or more than it. The genetic quality of sperm, motility of sperm & semen volume all typically decrease with age. Advanced paternal age is associated with more DNA damage, less apoptosis, and lower sperm motility⁶.

The object of this study was to correlate the adverse effect of advanced maternal age in clinically diagnosed patients of DS, further confirmed by cytogenetic study to know the type of trisomy.

2. Materials & methods

For the present study, 30 clinically diagnosed DS patients were selected. Their detailed clinical history was taken & clinical examination was done. To obtain their karyotype, about 0.8 ml of venous blood was collected in heparinised vacuttes. Culture setting was done using freshly tapped blood & put it in incubator at 37 degree for 72 hours. After that harvesting has been done & finally the metaphases were obtained on the slides. Then those slides showing metaphases with good morphology were selected and kept under dry wooden boxes for aging. After 7 days, banding procedure was done using Giemsa, freshly prepared Trypsin and EDTA solutions. The metaphase plates were observed in each case and finally, a photograph was obtained from a good quality metaphase slide with the help of a black and white film loaded camera attached with a photomicroscope with an exposure time of 8-15 seconds. The chromosomal findings were described according to the international system of Human Cytogenetic Nomenclature and finally, Karyotype was prepared using conventional cut and paste technique.

3. Results

In the present study of 30 cases of DS patients, mental subnormality was found in 92.3% of cases, hypotonia in 69.2%, mongoloid facies in 84.6%, prominent epicanthal folds in 73.1%, protruded tongue in 57.7%, simian crease in 61.5% & sandle sign was found in 42.3% of cases [Table-1].

Table 1: Distribution of clinical features in DS patients studied.

Clinical features present	Number of patients
Mental subnormality	28(92.3%)
Hypotonia	21(69.2%)
Mongoloid facies	25(84.6%)
Epicanthal folds	22(73.1%)
Protruded tongue	17(57.7%)
Simian crease	18(61.5%)
Sandle sign	13(42.3%)

The cytogenetic evaluation was done by karyotyping & were as follows:

Table 2: Cytogenetic findings in DS patients studied:

Total no. of patients	Trisomy 21	Translocation	Mosaicism	Normal
30	22(73%)	-	-	8(27%)

The above table shows that out of 30 clinically diagnosed DS cases, chromosomal abnormality was found in 73% of cases & 27% of cases were chromosomally normal. Out of 22 cases of chromosomal abnormality, all cases show trisomy 21. We had not found any single case of translocation &/or mosaicism in our study [Table-2].

Table 3: Maternal age distribution in DS patients studied.

Maternal age group(in years)	<20	21-24	25-29	30-34	35-39	Total
Number of cases	3(10%)	4(13.3%)	4(13.3%)	6(20%)	13(43.4%)	30

Above table shows that out of 30 patients studied, 43.4% of cases were in maternal age group of 35-39 years, 10% in age group of <20 years & 13.3% in age group of 21-29 years & 20% in age group of 30-34 years [Table-3].

4. Discussion

Down syndrome is caused by a gene dosage-imbalance resulting from human chromosome-21 trisomy, and is the most commonly diagnosed congenital malformation/mental retardation syndrome⁷. In 1866, John Langdon Down made the first detailed description of all affected individuals.

The overwhelming majority of DS birth is caused by trisomy 21 due to NDJ, failure of chromosomes to separate properly during meiosis at parental gametogenesis⁸.

Penrose identified advanced maternal age as risk for DS birth(1934) & postulated that in some way it is associated with NDJ^{9,10}. Antonarakis in 1991; Ballesta in 1999; Muller in 2000 found that NDJ effect is restricted only in the oocyte¹¹.

The maternal versus paternal basis for meiotic errors are well illustrated by their physiological timeline. Female meiosis I(MI) is initiated during fetal development, but after homologous recombination, the oocytes undergo a period of arrest in prophase. MI is then resumed (10–50 years later) in the ovary just before ovulation^{12,13}. After completion of MI, the oocytes are suspended in the metaphase of meiosis II (MII) and the second division is completed only after fertilization. The prolonged meiotic arrest phase likely allows accumulation of toxic effects including environmental insults, degradation of meiotic machinery causing MI and MII errors, and suboptimal ovarian functioning likely resulting in hormonal imbalance¹. Male meiosis, however, begins at puberty and all events are sequentially completed without interruptions, in the adult testis^{12,13}.

4.1: Biological aging hypothesis:

The hypothesis was proposed by Brook(1984)¹⁴. The central idea of this hypothesis is that the increasing rate of meiotic errors & subsequent aneuploid birth is related to ‘biological aging’ of ovary rather than chronological age of women. Two views explain how the biological aging is implicated for increased incidence of trisomic birth. The first view relates the suboptimal level of hormonal signal with higher rate of meiotic errors in aging ovary. The number of antral follicle at various stages of development also declines with increasing maternal age which along with decrease in total oocyte pool generates an imbalance in the hormonal environment in ovary predisposes the women for aneuploid conception¹⁵. The second view relates ‘limited oocyte pool’ which suggests a more direct effect of antral oocyte pool size on the risk of aneuploidy. Among older women available antral follicles are limited & ovary has to compromise in selecting a suboptimal or erroneous oocyte for ovulation¹⁶. The ‘biological aging’ can also be interpreted in term of senescence associated degradation of ovarian protein components that are implicated in chromosome separation system in oocyte¹.

4.2: Genetic aging hypothesis:

‘Genetic aging’ hypothesis was proposed by Ghosh, which states that some of the mothers having DS baby are genetically older than the mothers of same chronological age having euploid baby & genetic aging is the underlying cause of biological aging in ovary. According to this hypothesis with increase of age there is shortening of telomere length & lastly there is loss of telomere. He proposed that older women who have DS baby are “genetically older” than other mothers who have euploid babies at the same age. The fact of telomere shortening among women with DS child can be explained by a possible functional link between telomere maintenance system & chromosome segregating apparatus at molecular level. Degradation of this ‘molecular link’ with age may affect the both system simultaneously. Alternatively, the environmental factor that induces rapid telomere loss at advanced reproductive age might affect chromosome separation system in

oocyte¹⁷.

4.3: Reduced meiotic recombination & its interaction with maternal age:

Aside from maternal age, only single factor that has been identified unambiguously to be associated with maternal NDJ is altered pattern of meiotic recombination. The first evidence for association of reduced recombination with events of NDJ of chromosome 21 was provided by Warren(1987). Chiasmata are physical connections between homologous chromosomes at site of recombination & they function to stabilize the paired homologues or tetrad at MI along with sister chromatids & centromere cohesion¹⁸. It aids in proper chromosome orientation on meiotic spindle & ensure their proper segregation to opposite poles. Absence of chiasma formation left the homologous pair free to drift randomly to poles & if they move together to the same pole aneuploidy results. As far as chromosome 21 NDJ is concerned, achiasmatic meiosis is the major cause of reduction in recombination frequency¹⁹.

Among the young mothers risks related to aging is minimum & therefore absence of recombination becomes the predominant cause of NDJ in total risk scenario. If this remains true, then lack of recombination is an age-independent risk factor for chromosome 21 NDJ. In human there is existence of proteins that act as surveillance system to ensure proper segregation of non-exchange meiotic chromosomes. Age-dependent down-regulation of these essential proteins may lead to decreased ability to segregate properly the non-exchange chromosomes in aging oocyte.

A single telomeric chiasma is a risk for malsegregation of chromosome 21 at MI in oocyte in contrast to single pericentromeric chiasma which increases risk of MII NDJ. Single telomeric exchange is prevalent among younger mothers whose chromosome 21 nondisjoined at MI. In contrary, single centromeric chiasma is risk for MII NDJ particularly at older age¹⁹.

One of the members of centromeric cohesion complex shugoshin, when down regulated due to aging shows high frequency of MII NDJ of bivalent with pericentromeric exchange²⁰.

The consequence of increased proximal crossovers contributing to MII errors may be due to (a) chromosomal entanglement at MI, wherein the bivalents are not separated until MII, or (b) premature separation of sister chromatids at MI, due to loss of sister chromatid cohesion¹⁹. Here, there is separation of the whole chromosome and a single chromatid to each pole of the meiotic spindle¹⁹. Notably, there are several other possible patterns of MI and MII errors^{12,13}.

5. Summary & Conclusion

We have paved half of century after the initial discovery of cause of DS, but we are still in dark regarding etiology of DS. It has been identified that advanced maternal age is a risk factor for DS. With advanced maternal age deteriorating hormonal levels, decreased numbers of healthy oocyte for fertilization, telomere loss & reduced meiotic recombination all leads to increased susceptibility for DS. Recently noticed that gene PRDM9 that controls recombination, its variant has been reported to make the women susceptible for recurrent miscarriages, infertility & aneuploidy births. The gene BubR1 a member of centromere cohesion complex is responsible for cellular aging. Environmental aneugens tend to be accumulated within ovarian microenvironment during protracted oocyte growth phase & lead to adverse effects. The periconceptional smoking & contraceptive use have been identified as potential risk for chromosome 21 NDJ. Therefore we realized that the risk factors associated with DS birth is multidimensional & several mechanisms are involved for chromosome 21 NDJ in women.

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