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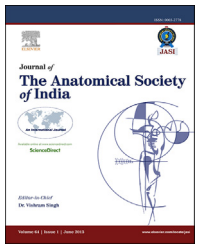

Original Article

Cytogenetic variations in a series of cases of Down Syndrome

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ABSTRACT

Introduction: Down Syndrome (DS) is generally associated with mental retardation and developmental delay. The occurrence of DS is associated with multiple factors like maternal age, consanguineous marriage, early induced abortion etc. Chances of recurrences of DS in next pregnancy depend on the genetic constitution of the affected individual and the parents. So this study was done to find out the different types of cytogenetic abnormalities in DS patients and also the association of parental age to DS in a population in West Bengal. It is hoped that the present study will emphasize the need for genetic counseling of prospective parents as well as parents of individuals affected with DS, together with cytogenetic screening of pregnancies which are at high risk for DS.

Methods: A cytogenetic analysis was performed using conventional GTG banding on 120 patients with clinical features of DS, referred to the Department of Genetics, Vivekananda Institute of Medical Sciences, Kolkata during the period from October, 2008 to September, 2014.

Results: Cytogenetic analysis confirmed the diagnosis of DS in 117 cases, among them regular trisomy constituted 83.76%, mosaicism recorded in 11.11% and Robertsonian translocation in 5.12% of cases. The mean maternal age was higher in regular trisomy 21 (25.08 yrs) than in translocation (22.50 yrs). No significant difference was noted in mean paternal age among different categories of DS cases.

Discussion: This study documents the types of cytogenetic abnormalities in DS children of West Bengal population of India and thus emphasizes the need for genetic counseling in these cases.

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1. Introduction

Down Syndrome is the commonest autosomal genetic disorder in human with a prevalence of 1:600 newborn.[1] The prevalence of Down Syndrome (DS) in India is 0.88 per 1000 (1 out of 1139) to 1.09 per 1000 (1 out of 916) and three DS children are reported to be born every hour.[2,3] Patients with DS have a characteristic phenotype along with mental retardation and developmental delay. Although there is a considerable variation in the appearance of individuals with DS, they present a constellation of features that help the clinician to make a diagnosis. The facial features include a low nasal root, upslanting narrow close set palpebral fissures, measurably small and sometimes over-folded ears and a flattened maxillary and malar region with irregularly arranged teeth and large furrowed semi-protruded tongue giving the face a typical appearance. Males have poorly developed genitals and are almost always sterile. In females, ovarian defects and irregular menstruation are the rule, but fertility is possible and over two dozen live births have been recorded. They may also present with heart disease (40–50%) and duodenal atresia, increased risk of acute myeloid leukemia, Hirsprung's disease and Alzheimer's disease (especially after the fourth decade).[4] Diagnosis is evident from typical clinical features but the cytogenetic abnormalities should be studied for determination of the risk of recurrence and thereby helping in genetic counseling.

DS presents mainly in four cytogenetic forms[5,6]:

1. **Regular trisomy 21** is due to meiotic non-disjunction (T21) having karyotype 47,XX,+21 or 47,XY,+21 present in 93–95% of cases.
2. **Robertsonian translocations (RT)** involves the rearrangement of chromosome 21 with another acrocentric chromosomes (Group D or G), 46,XX, or 46,XY,rob(D or G;21)(q10;q10), they present in approximately 4% of cases.

D group includes 13, 14, 15 chromosomes, G group includes 21, 22 chromosomes.

3. In **Mosaicism** there is presence of two or more different cell lines in the same individual. In these cases, one line with T21 with another normal or abnormal line, represented by the formula 47,XX or XY,+21/46, XX or XY & correspond to 1–3% of all cases.
4. **Non-classical forms** like partial trisomy of the region 21q22.3 with karyotype 46,XX or 46,XY,dup(21)(q22.3), trisomy 21 associated with other chromosomal disorder – observed in <1% cases etc.

There are several reports on the increased incidence of DS from the different parts of the world with respect to ethnicity and parental age.[7,8] Prenatal screening is still inaccessible to most families in developing countries like us and almost all patients were diagnosed during postnatal period.

This study was thus conducted to document the prevalence of cytogenetic variants of Down Syndrome in West Bengal population and their relation to parental age.

2. Materials & methods

The study included 120 children in the age range of 4 days–15 yrs with phenotypically suspected Down Syndrome. They were referred to Vivekananda Institute of Medical Sciences (VIMS), Kolkata during the periods of October, 2008 to September, 2014 from different areas of West Bengal. This study was approved by the ethics review board of VIMS.

The blood sample was collected from the patients in a completely sterile heparinized vacutainer tube and mixed well. The cultures were set up with RPMI 1640 (Rosewell Park Memorial Institute) culture medium. Peripherial blood lymphocytes inducted with 2% phytohemagglutinin (PHA) were incubated at 37.5 °C for 72 h. One and a half hours prior to harvest, the cultures were arrested with colchicine and treated with 0.75 M KCl (potassium chloride) for 30 min and fixed in 3:1 ratio of methanol/glacial acetic acid fixative. After air drying, routine Giemsa (GTG) banding technique was performed to identify the chromosomes. After banding, 50 metaphases were scanned under low power for each case on OLYMPUS BX51 microscope and then 10 metaphases were analyzed by automated karyotyping system (CYTOVISION software). In cases of mosaics 30 metaphases were analyzed.

3. Results

Among 120 cases of phenotypically DS three showed normal karyotype. In rest of the 117 cases the chromosomal patterns are presented in Table 1. Free trisomy 21 was found in 98 cases (83.76%) (Fig. 1) and 13 cases (11.11%) showed mosaicism. In mosaicism group three cases of trisomy 21 along with Robertsonian translocation were noted, rest mosaics showed trisomy 21 along with normal cell line. In 6 cases (5.12%) pure translocation was noted (Figs. 2 and 3).

The majority of Down Syndome patients belonged to the age group of 4 days–15 years. The mean age of referral didn't differ in different categories of karyotypic abnormalities. Only few patients were referred from the neonatal ward while the others were referred for delayed development and speech defect.

The mean parental age in different type of DS is shown in Table 2. From the table it is evident that mean maternal age is lower in Robertsonian translocation group than trisomy 21 group. In our study no significant relation was found with the paternal age in the occurrence of DS.

4. Discussion

Aneuploidy is the most common and clinically significant type of human chromosome abnormality, occurring in at least 3–4% of all clinically recognized pregnancies.[4] Down Syndrome or Trisomy 21 is the most common aneuploidy in live born fetuses and is associated with mental retardation and developmental delay.

Essentially, DS consists of three or more copies of the genetic material of chromosome 21. This may occur as 3 copies

Table 1 – Cytogenetic variants found in DS cases in the study population.

Karyotype	No. of cases	Percentage (%)
Regular trisomy 21		
47,XY,+21	57	83.76
47,XX,+21	41	
Mosaic		
47,XX,+21/46,XX	6	11.11
47,XY,+21/46,XY	4	
47,XY,+21/46,XY,rob(21;21)(q10;q10)	1	
47,XY,+21/46,XY,rob(14;21)(q10;q10)	2	
Robertsonian translocation		
46,XX,rob(14;21)(q10;10)	2	5.12
46,XY,rob(21;21)(q10;10)	4	
Total	117	100 (approx.)

of the entire chromosome 21, or portions of its long arm. Parts of the long arm of chromosome 21 thought to be responsible for clinical DS are collectively known as Down Syndrome Critical Region (DSCR). This region is a single gene or gene clusters, many of which lie near the tip of the long arm in bands 21q22.13–21q22.2 whose duplication is largely responsible for most of the DS phenotypic features.[9] On the other hand, some cases of DS are mosaics, having a mixture of trisomy 21 cells and normal cells but the minimum percentage of trisomic cells required to manifest DS has not yet been established.

In our present study we have encountered different karyotypic variations of Down Syndrome.

Meiotic non-disjunction occurs in approx 95% cases of DS which results in trisomy 21 (T21), the most frequent cytogenetic variant of DS. Non-disjunction occurs due to failure of either of two homologous chromosomes to pass to separate cells during the first meiotic division, or of the two chromatids of a chromosome to pass to separate cells during the second meiotic division. As a result, one daughter cell has two chromosomes or two chromatids and the other has none.

In our present study the frequency of free T21 observed was maximum and in seen in 83.76% cases which is consistent with the other study done in Indian population.[10,11] Several researches were conducted to find out the cause of interference of normal occurrence of biochemical events in DS individual.

Occurrence of Mosaicism in small fraction of cases of DS may be due to mitotic non-disjunction in the embryo rather than to meiotic non-disjunction in either of the parent. It may occur during the second cleavage division of the zygote or at a later stage of cleavage division producing an embryo with normal and trisomic cells and initially one monosomic cell line; subsequently the later usually dies. So this embryo will be a mosaic and will most likely develop into an individual with some clinical features of DS. Incidence of this condition in our study was higher (11.11%) than that reported in the earlier studies,[5,10–12] but consistent with the study by Chandra N et al.[13] Down Syndrome patients with mosaicism with a trisomic cell line in a proportion >90% are diagnosed as regular trisomy because unless analyzing >50 cells, normal line is not detected. On the contrary, if the trisomic line is in a proportion of <10%, the diagnosis often goes unnoticed.[14] With the help of advanced technology of CYTOVISION software we could analyze on average 30 cells per case which helped us to find out the exact prevalence of mosaicism in this population. These mosaic individuals depending on what proportions of tissues end up being trisomic and which specific tissues are these, show the clinical features. The earlier the non-disjunction takes place, the larger the proportion of aneuploid cells that might be found in the mosaics. Those individuals with only a tiny proportion of aneuploid cells may have a completely normal phonotype and escape detection entirely. Thus it is considered that the reported frequency of mosaicism in cases of DS is lower than the true frequency. When mosaicism is suspected or detected, it is recommended to look for the trisomic line, in at least two tissue samples. It has been observed that the number of abnormal cells in the oral mucosa is significantly related to I.Q (cells derived from ectoderm). In contrast, cardiac defects correlate with the proportion found in lymphocytes because both tissues derived from mesoderm.[15] In our study we have not verified mosaicism in other tissues, but this factor is to be included in future studies.

In non-disjunction and mosaicism, recurrence rarely occurs in siblings. But in some families with multiple cases

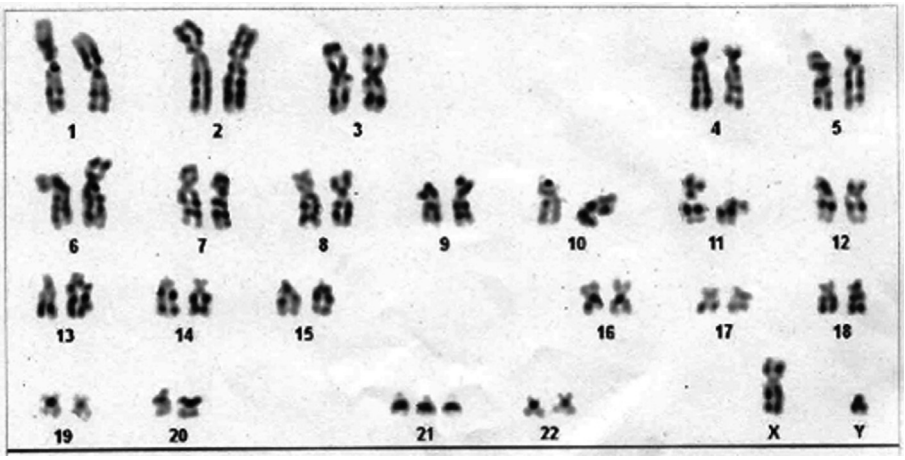

Fig. 1 – Karyotype of pure trisomy Down Syndrome.

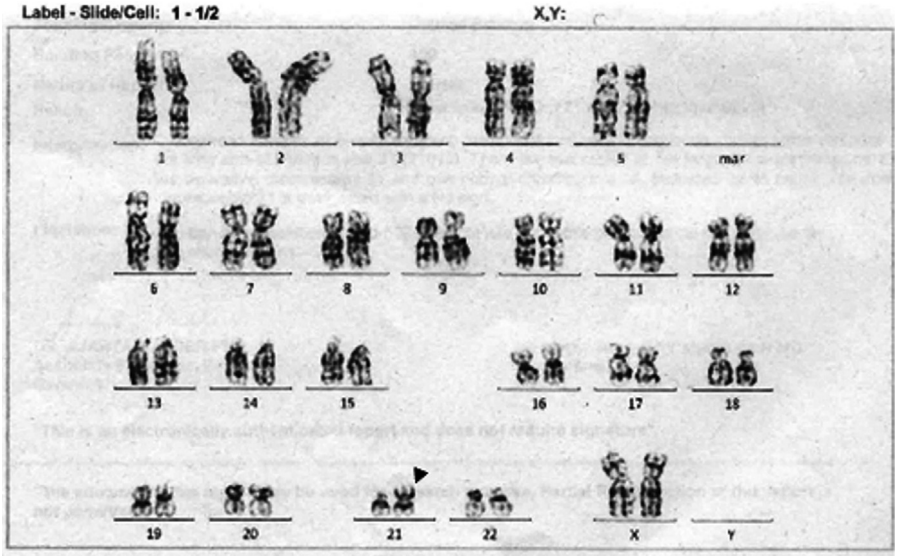

Fig. 2 – Karyotype of 46,XX,rob(21;21)(q10;10).

of trisomy, aneuploidy was found in a mosaic parent's gonadal tissue. These instances of germinal mosaicism should be detected early, because unlike the usual sporadic pattern, the occurrence of non-disjunction in the mosaic parent's trisomic germ cells can lead to multiple DS births in a family.[16]

Robertsonian translocation is another cause of DS and it takes place when the long arms of two acrocentric chromosomes 13, 14, 15, 21 and 22 (D & G groups) join. Carriers of these translocations do not present with any disorder themselves, yet cause unbalanced chromosomal formation during parental gamete formation. In case of DS, RT occurs mainly between two 21 or between 14 and 21 chromosomes. At the process the participating chromosomes break at the centromere, and lose their short arms. The long arms join and form a single chromosome with a single centromere or two. The remaining short arms fuse as well and form a reciprocal product which is lost in time within a few cell divisions. These balanced rearrangements do not alter the amount of genetic material, so the carriers of these rearrangements are healthy, normal at phenotype but unaware of the possible results of conception which are recurrent fetal losses, infertility and births with abnormal phenotype due to abnormal segregation during meiosis. In our study it comprised of 5.12% of DS cases and this is higher than the percentage found in other similar studies in India, which were 3.8%[9] and 4.4%.[10] Familial inheritances in RT is seen in one fourth and usually the mother is the carrier whereas in remaining it arises de novo. So when a patient with DS has one of these variants it is necessary to conduct karyotyping of the parents to identify if one is a

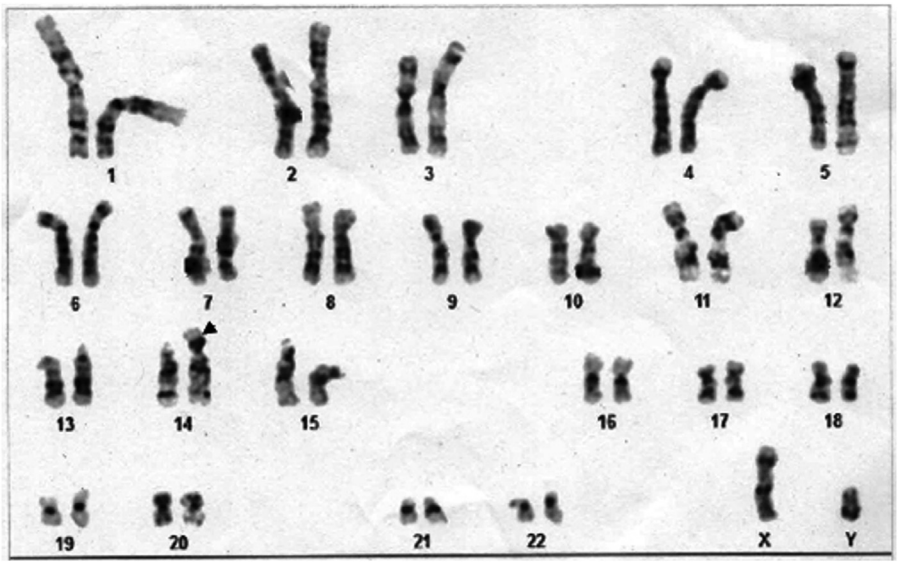

Fig. 3 – Karyotype of 46,XY,rob(14;21)(q10;q10).

Table 2 – Parental age distribution in different cytogenetic variants.

Mean age (yrs)	Trisomy 21	Translocation	Mosaicism
Maternal	25.08	22.50	22.78
Paternal	31.09	30.44	29.50

carrier and to establish an appropriate risk of recurrence in next pregnancy.[17] Mother of a translocated DS may be a carrier of D/G group chromosome translocation with only 45 chromosomes. She may produce different types of gametes, one with normal D and G chromosome, one with translocated D/G chromosome causing a balanced carrier, one with D/G chromosome and normal D chromosome, one with normal G chromosome, one with normal D chromosome and one with D/G chromosome and normal G chromosome (Fig. 4). The offspring derived from the last variety of gamete will have 46 chromosomes but will be trisomic for chromosome 21 with DS. Therefore, a carrier mother with D/G translocation will have a risk of getting a child with DS. The risk of recurrence is <1% if the translocation is de novo. In case of familial RT (Robertsonian translocation) DS, the genetic risk for rob(D,21) female carrier to have a foetus with translocation DS is 15% at the time of detection by amniocentesis and to have a live born child with translocation DS is about 10%; for male carrier the recurrence risk is about 2–5%. For a carrier of a rob(21,21) the risk for recurrence is 100%.[18] In our study only in two cases it was possible to study the karyotype of the parents and in both cases translocation was found to be de novo in origin. In other cases of translocation DS, parents were counseled to have their karyotype done prior to their next pregnancy; or to do chorionic villous sampling during early stage of next pregnancy.

In the last few decades, cases of non-classical DS karyotype have been reported in major DS studies with frequency 0–1.2%.[8] Frenny J. Sheth in their study revealed incidence of inv (Y) in DS cases of Gujrat as 1.67%.[19] Chandra N, et al,[13] Parihar M, et al[20] found mosaic double aneuploidy in DS patients (47,XY,+21/47,XYY) which is a very rare entity. In our study population we didn't find such atypical karyotype. It is important to consider such non-classical DS cases in genetic counseling and provide precise recurrence risk for such distinct groups.

In 3 cases we have found normal karyotype, in spite of having typical phenotype of Down Syndrome. It would be desirable to perform molecular studies such as Fluorescence in situ hybridization (FISH) with probe for the 21q22 region in different tissues in order to rule out the low proportion of mosaicism or cryptic rearrangements in these cases.

Though the advanced maternal age is an established risk factor for DS, present study has shown increased number of DS babies born to the younger mothers, in the age group 21–25 years (32%), whereas the lowest number belonged to mothers in the age group of >40 years (Fig. 5). This finding is clearly related to the greater number of pregnancies in this group. However, in general, the prevalence of free trisomy is increased significantly in women older than 35 years; this could either be due to MTHFR gene polymorphism and/or nutritional factor.[11,21] In case of translocation DS all the mothers were below 26years. In our study maximum numbers of fathers were in the age group of 24–35 years (average age being approximately 31 years) and no correlation of

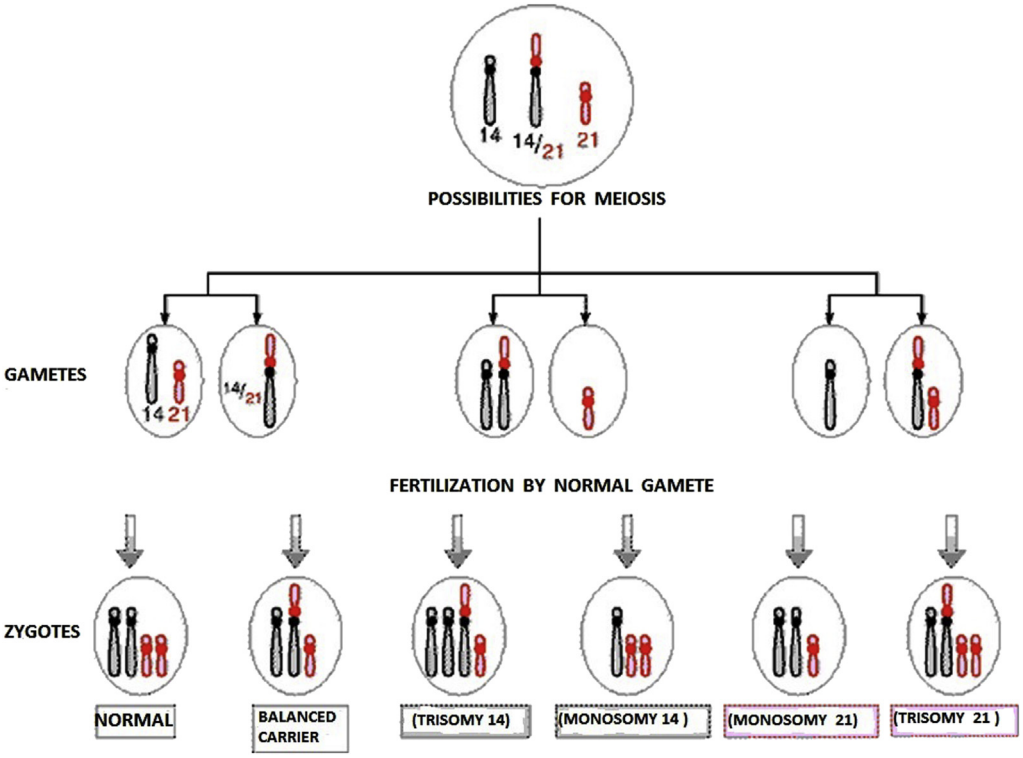

Fig. 4 – Possible gamete formation by a carrier mother of balanced translocation.

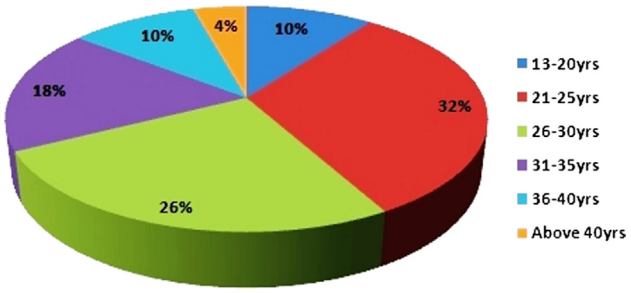

Fig. 5 – Age distribution of mothers having Down Syndrome babies.

occurrence of DS was found with paternal age similar with other previous studies.

5. Conclusion

Our study showed higher occurrences of mosaic DS in local population which may be due to more accurate identification of abnormal karyotype by the use of cytovision software. It also showed increased incidence of Robrtsonian translocation in the study population which re-enforces importance of cytogenetic analysis of DS patients to calculate the risks for recurrence and to provide genetic counseling.

Conflicts of interest

All authors have none to declare.

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