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# Increased Frequency of Down Syndrome in Young Mothers of Rural Population

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***Abstract***

Studies were conducted in 222 children clinically diagnosed with Down syndrome (properly diagnosed on the basis of symptoms by Dr. Chandraleka, Pediatrician) to understand the frequency and type distribution of such cases; and to determine the origin of extrachromosome in the 21st pair. Clinical diagnosis was confirmed with karyotyping. Results have shown that nondisjunction or pure trisomy 21 appears to be the main cytogenetic cause; with a percentage frequency of 96.4%; comprising of 114 boys (53.3%) and 108 girls (46.7%). Also the cytological and molecular analysis result revealed that Down syndrome was increased in the young Indian mothers especially in the rural areas.

## 1. Introduction

Down syndrome is the most common, easily recognized and probably the most researched chromosomal disorder characterized by varying degree of mental retardation and other multiple defects. It is caused by an extra chromosome 21 in the G group; or in a small percentage of cases, by the translocation of chromosome 21 on to chromosomes 13, 14 or 15 (D group) and chromosomes 21 or 22 (G group). The parental prevalence of the disorder is much higher; as only 70% of fetuses identified as Down syndrome in mid trimester survive to full term [1]. It has been estimated that trisomy 21 occurs on 0.45% of conception; but more than 75% of these do not survive to full term [2].

All individuals with Down syndrome have the extra critical portion of chromosome 21 (region 21q / 22 along the distal segment of the long arm) and occurs in the form of trisomy 21, translocation or by mosaicism. Among these trisomy 21 is the most common form; mounting upto 95% of cases [3]; and that too are mostly due to maternal meiotic non-disjunction event [4]. Incidentally trisomy is the most common chromosomal abnormality in humans and affects 4 % of all clinically recognized human pregnancies [2]; and trisomy 21 is the most frequent live born aneuploidy [5].

The only well established risk factor for Down syndrome is advanced maternal age [6]; may be related to the physiological status of ovaries [7]. Some reports suggest advanced paternal age also contribute to this condition [8]. Some evidence suggests that thyroid disorders in the mother may increase the risk of bearing a Down syndrome child [9]. Abnormal folate metabolism and mutations in the methylene tetrahydrofolate reductase and methionine synthase reductase genes have been attributed to be causative factors [10].

However, the data of human molecular genetics and the development of physical map of chromosome 21 are confusing. Therefore, the present study is undertaken to recognize the molecular markers and to assess the prevalence of the phenotypic character of the Down syndrome as phenotypic maps can provide the basis for clinical prognosis for individuals with partial aneupoidy for chromosome 21.

## 2. Materials and Methods

### Patients

Two hundred and twenty two subjects with Down syndrome were investigated in the Genetics and Birth Defects Clinic, Institute of Child Health (ICH) hospital for children, and Marthru Mandir, Downs Syndrome Association of Tamil Nadu (Day care centre for Down Syndrome Children) Chennai. A complete clinical assessment, family history and pedigrees were recorded in special case proformas.

### Method

The peripheral blood lymphocytes were used for chromosomal studies using G-banding [11] and high resolution banding method [12]. About 2-3 ml sodium heparinized blood was collected, and lymphocytes were grown in TC 199 and Ham F10 culture media, along with fetal calf serum, phytohaemoagglutinin and antibiotic (penstrep), followed by incubation at 37°C. At the end of 69th

hour, cells were arrested in metaphase using colchicine, and further incubated for 45 minutes at 37°C. Hypotonic treatment was given to the centrifuged pellet with KCl (0.75M) and further incubation for 20 minutes at 37°C in water bath. The cells were then washed and fixed with Carnoy's fluid (3:1 Methanol and Glacial Acetic acid) [13]. Slides were prepared by dropping the pellet suspended in a fixative on chilled precleaned slides, placed at 50°C on the hot plate and dried. This was followed by trypsinization treatment (0.1-0.5% (Trypsin EDTA), stained by Giemsa stain for 7-8 minutes, washed under running tap water, subsequently air-dried. The G-banded metaphases were then analyzed under oil immersion lens (100X) using Carl-Zeiss microscope.

Twenty patients and their parents were genotyped for markers located on chromosome 21. DNA was extracted from white blood cells in the peripheral blood samples and PCR was used to detect chromosome 21 polymorphisms. Five microsatellite markers were used to identify the parental origin as described by Sherman et al [4].

## 3. Results and Discussion

Chromosomal analysis was done on 222 clinically diagnosed Down syndrome patients. The karyotype analysis revealed 96.40% (214 of 222 cases) with trisomy 21, i.e. having 3 copies of chromosome 21; in 2.7% (6 cases) translocation was observed, i.e. 1 copy was translocated to another acrocentric chromosome; and in 0.90% (2 cases), there was a mosaicism as shown in Table 1.

### Maternal age and Down syndrome

Free trisomy 21 (nondisjunction) was found in 87.5 % with maternal age between 15-20 years (Group I); 96.8 % between 21-34 years (Group II), and 100 % above 31 years of age (Group III) (Table 2). Translocation was found higher (12.5%) in group I when compared to group II (1.87%). Mosaicism was found only in the Group II (1.25%).

### Non-disjunction (Trisomy 21)

It has long been recognized that the risk of having a child with Down syndrome increases with maternal age [14]. The increase in risk for chromosomal abnormalities with relation to women age is gradual until the age of 33, after which the risk begins to rise at a faster rate [15]. Chromosomal non-disjunction is a random event that occurs more frequently as women get older. However, since it can occur at any time, children with trisomy 21 can be born to women of all ages. In fact, because most pregnancies occur in younger women, approximately 80% of all babies with trisomy 21 are born to women under the age of 35 [16]. In the present study, 82% had maternal age less than 30 years. Thus, offering the evidence that young maternal age increases risk for a nondisjunctional event in the ovum.

### Translocation

In the present study only 6 (2.7%) cases had Down syndrome resulting from Robertsonian translocation. The translocations most commonly involve a D group chromosome [17]. Translocation occurs 10% of the children born to mothers between 15 and 19 years of age [18]. In our study 50% (3 out of 6) translocation was seen between 15 and 20 years of maternal age, and 50% between 21-30 age group (Group-II).

### Mosaicism

Mosaic (46/47, +21) is detected in 2% to 3% in Down syndrome [19, 20]. The present study population having a preponderence of Down syndrome had demonstrated a lower incidence (0.9 %) of mosaicism in Down syndrome (Table 1). The common concept is that advanced maternal age (> 35 years) is at an increased genetic risk for Down syndrome baby. Since, most pregnancies in our country occur in younger women, trisomy 21 children are therefore born to women under the age of 35 years as shown in the present study (Table 2). The mean maternal age was raised in free trisomy 21, but not in translocations [21] as evident in the present study. In India, most pregnant patients have already passed the period of fetal development by the time they first seek antenatal care. Consequently to promote perinatal safety, it is necessary to identify those who are at risk and then to prevent mortality and morbidity.

Table 1 Genetic profile of 222 Down syndromes

| S. No. | Cytogenetic profile | No. | % |
|---|---|---|---|
| 1 | Free Trisomy (Nondisjunction) | 214 | 96.40 |
| 2 | Translocation | 6 | 2.70 |
| 3 | Mosaicism | 2 | 0.90 |

Table 2 Correlation of maternal age and chromosomal aberration

| Group No. | Age range | N=222 | Cytogenetic Profile | No. | % |
|---|---|---|---|---|---|
| I | 15-20 | 24 | Free trisomy | 21 | 87.5 |
| | | | Translocation | 3 | 12.5 |
| II | 21-30 | 160 | Free trisomy | 155 | 96.8 |
| | | | Translocation | 3 | 1.87 |
| | | | Mosaicism | 2 | 1.25 |
| III | Above 31 | 38 | Free trisomy | 38 | 100 |

**Maternal abortion and birth of Down syndrome offspring**

Among the Down syndrome families (222), only once spontaneous abortion occurred in 59 mothers before the birth of DS (26.58%), twice in 7 mothers (3,15%), four times in a single mother (0.45%) (Table 3). Buck et al. [22] were the first to point out that "if a group at risk for nondisjunction existed, then one might find an increased incidence of fetal loss in their obstetric history". The relative risk to produce a DS child is associated with an increase in the number of abortions in younger women [23]. A history of spontaneous abortion may identify a group of women at risk to get a trisomy child. In the present study the high risk group seems to be women whose age ranges from 21-25 years.

Table 3. Spontaneous abortion in relation to marriage age of mothers

| S.No. | Age | Number of spontaneous abortion | | | | Total (222) |
|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 4 | |
| 1 | 15-18 | 40 | 11 | 1 | 1 | 53 |
| 2 | 19-20 | 43 | 8 | 1 | - | 52 |
| 3 | 21-25 | 47 | 31 | 5 | - | 83 |
| 4 | 26-30 | 19 | 6 | - | - | 25 |
| 5 | 31-35 | 6 | 3 | - | - | 9 |

**Paternal occupation and birth of DS**

Occupation of the fathers of DS indicated that 61 subjects were coolies / daily wagers (27.48%), 28 subjects were farmers (12.61%), 31 subjects were business people (13.96%), 36 subjects were technicians (16.22%), 46 subjects were office workers (20.01%) and 20 subjects were other employees (9.01%) (Table 4). This study found certain paternal occupations to be associated with an increased risk of having a child with Down syndrome.

Table 4. Paternal occupation and chromosomal aberration

| S. No. | Occupation of father | N=222 | % |
|---|---|---|---|
| 1 | Daily wagers | 61 | 27.48 |
| 2 | Farmers | 28 | 12.61 |
| 3 | Business Men | 31 | 13.96 |
| 4 | Technicians | 36 | 16.22 |
| 5 | Office Workers | 46 | 20.01 |
| 6 | Others | 20 | 9.01 |

**Molecular analysis - Genotyping**

DNA polymorphic analysis was done to determine the parent and meiotic stage of origin of the extra chromosome 21 and assessed the frequency of recombination between the nondisjoining chromosomes in 20 families. The hybrids were screened using five microsatellite markers D21S214, D21S232, D21S213, IFNAR and D21S171 to infer the presence of a centromere and at least one in proximal, medial and distal 21q. DNA polymorphic results revealed that all twenty of trisomy 21 cases were maternally derived, 70% of the errors occurred during meiotic I (MI) and 30% occurred during meiotic II (MII). Among them 25% MI appeared in the age group of 15-20; 30% of MI and 20% of MII appeared in 21-25 age group and 15% of MI and 10% of MII appeared in the age group of 26-30 (Table 5).

Table 5. Effect of age of mothers on the meiotic division towards DS

| Age of mothers | Meiotic I (MI) | | Meiotic II (MII) | | | |
|---|---|---|---|---|---|---|
| | Frequency | % | Frequency | % | Frequency | % |
| 15-20 | 5 | 25 | 0 | 0 | 5 | 25 |
| 21-25 | 6 | 30 | 4 | 20 | 10 | 50 |
| 26-30 | 3 | 15 | 2 | 10 | 5 | 25 |

Our data suggested that young Indian mothers in the rural areas are associated with birth defects such as non-disjunction, abortion etc. These observed changes may be due to the nutritional deficiencies or because of environmental factors like high stress related conditions. As women carrying fetus of Down syndrome have been found to have low maternal serum levels of alpha feto protein and estrial and elevated levels of human chorionic gonado protein [24], prenatal screening of these substances along with chronic villus sampling and amniocentesis, have made it possible to identify cases of Down syndrome in utero. As a result, in many countries where elective termination is practiced, the birth prevalence of Down syndrome is reduced [25, 26]. However in India such practices are just becoming available. Future studies could be focused on these specific, rather than general groups so that causative agents may be confirmed and thus enable appropriate preventive measures to be taken.

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