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Maternal Age and Chromosomal Profile in 160 Down Syndrome Cases – Experience of a Tertiary Genetic Centre from India

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KEY WORDS Down syndrome; maternal age; nondisjunction; translocation; mosaicism.

ABSTRACT The present study reports the correlation of maternal age and chromosomal aberration found in 160 Down syndrome cases. The clinical diagnosis of Down Syndrome was evaluated with reference to cytogenetic profiles and maternal age,which revealed that 79% of mothers were below the age of 35 years. The cytogenetic study was conducted using peripheral lymphocytes culture for G-banding and High Resolution Banding of chromosomes. The trisomy 21 due to disjunction effect of chromosomes was predominant as 88.75% of Down syndrome revealed free trisomy 21. Among the advanced (>35 years) age group of mothers, however it was 95.5%. As most pregnant women in our country, while seeking the antenatal or perinatal care are in the younger age group, the routine pregnancy monitoring in healthcare is emphasized as a preventive strategy.

INTRODUCTION

Down syndrome is a genetic disorder occurring in approximately 1 in 650 to 1000 live births (Hook, 1982). It is the most common genetic cause of mental retardation accounting for 25-30% worldwide (McLaren and Bryson 1987). John Langdon Down (1866), an Englishman published the first clinical description of Down syndrome and later, Waardenburg (1932) suggested that the syndrome was a consequence of a chromosomal abnormality. Lejeune et al. (1959) confirmed the presence of trisomy 21 in Down syndrome.

Cytogenetic studies have shown that 95% of Down syndrome cases are trisomic due to non-disjunction, 4% are trisomic due to translocation (i.e. chromosome 21 attaches to acrocentric chromosome, either 13, 14, 15, or 22), and 1% are mosaics. Non-disjunction is more common in children born to mothers above 35 years of age, while translocation is more common in younger mothers. The risk of having a Down syndrome child at maternal age 30 is 1 in 1000 and at maternal age 40 is 9 in 1,000 (Hook 1982; Hook et al. 1983).

The aim of the present study was to determine cytogenetic profiles of Down syndrome cases in correlation with maternal age, among referrals to a tertiary genetic centre. Although there are a few published reports on incidence of Down syndrome (Verma et al. 1991; Isaac et al. 1985), there are hardly any studies on Indian population ascertaining the maternal age.

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MATERIALS AND METHODS

Patients

Centre for Research in Mental Retardation (CREMERE), is a tertiary referral centre having a well developed human genetic laboratory and conducts cytogenetic and metabolic investigations. In addition to diagnostic facilities, psychological and speech assessment, management, therapy and genetic counseling are the other services provided to the patients with mental retardation, genetic disorders and / or other related conditions.

One hundred and sixty Down syndrome cases were clinically diagnosed by characteristic facial features, and were referred for cytogenetic studies. Thirteen parents of 160 cases also agreed to undergo karyotype tests.

Method

The peripheral blood lymphocytes were used for chromosomal studies using G-banding (Sumner 1982) and high resolution banding method (Yunis et al. 1979). The 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) (Brown and Lawce 1997). 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.

For higher resolution of chromosome bands (750-800), in comparison with routine G-banding (400-450), the Ethidium bromide was added at 68th hour and further processed as mentioned above.

RESULTS

Chromosomal analysis was done on 160 clinically diagnosed Down syndrome patients. The karyotype analysis revealed 88.75% (142 of 160 cases) with trisomy 21, i.e. having 3 copies of chromosome 21; in 2.5% (4 cases) translocation was observed, i.e. 1 copy was translocated to another acrocentric chromosome (chromosome no. 13 or 14); and in 8.75%(14 cases), there was a mosaicism for a trisomic and a normal cell line as shown in Table 1.

In 105 cases, maternal age was known. Free trisomy 21 (nondisjunction) was found in 75% with maternal age between 18-20 years (Group-I); 84% between 21-34 years (Group-II), and 95.5% above 35 years of age (Group-III) (Table 2). Translocation and mosaicism were observed relatively higher in Group-II when compared to younger and advanced age groups i.e. Groups I and III, respectively.

Table 1: Cytogenetic profile of 160 Down Syndromes

S.No.	*Cytogenetic Profile*	*No.*	*%*
1	Free Trisomy (Nondisjuction)	142	88.75
2	Translocation	4	2.5
3	Mosaicism	14	8.75

Chromosomal Studies of Parents having Down Syndrome Children

The Karyotype was possible in 13 couples of the 160 cases, due to the cost factor. Normal karyotype pattern was found in 7 cases; while the remaining 6 cases showed chromosomal pattern with variants not involving chromosome 21.

Three of 6 Down syndrome cases had inherited the chromosomal variant [inv 9, inv 10 and t(11;22)] from their parent besides having nondisjunction trisomy 21. The remaining 3 cases had free trisomy 21 only and had not inherited the chromosomal variants from their parents.

DISCUSSION

The data on 160 children with clinical diagnosis of Down Syndrome was analyzed, and correlation of maternal age with cytogenetic profiles was done. Free trisomy was found to be 88.75% (Table 1), slightly lower than the reported.

Non-disjunction Trisomy 21

It has long been recognized that the risk of having a child with Down syndrome increases with maternal age (Penrose 1933). 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 (Hook et al. 1983).

The American College of Obstetricians and Gynecologists (ACOG) recommends that all women above the age of 35 at the time of delivery be offered amniocentesis during pregnancy. Although the cut off limit of age 35 has been arrived at arbitrarily, it is still the traditional age

Table 2: Correlation of maternal age and chromosomal aberration

Gp.No.	*Age range*	*N=105*	*Cytogenetic Profile*	*No.*	*%*
I	18-20 years	8	Free trisomy 21 (ND)	6	75
		(7.6%)	Translocation	1	12.5
			Mosaicism	1	12.5
II	21-34 years	75	Free trisomy (ND)	63	84
		(71.4%)	Translocation	3	4
			Mosaicism	9	12
III	≥35 years	22	Free trisomy 21 (ND)	21	95.5
		(21%)	Mosaicism	1	4.5

at which a woman is considered to be at high risk for chromosomal abnormalities (Erickson 1978).

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 (Holmes, 1978). It is very much evident in this study as 83 cases (79%) had maternal age between 18-34 years of age (Groups I and II together). This makes up 79% women to be less than 35 years. Earlier Indian study reported that 76.6% of Down syndrome are born to women less than 30 years of age ((Kaur and Verma 1951). In the present study, 60% had maternal age less than 30 years .

The chromosomal profiles of Down syndrome cases having maternal age ≥35 years showed 95% non-disjunction, which is greater than Group-II (84%) and Group-I (75%). Thus, offering the evidence that advanced maternal age increases risk for a nondisjunctional event in the ovum (Erickson 1978). Various hypotheses put forward to explain non-disjunction of chromosome 21 are -

- Intrinsic ovarian aging in advanced maternal age predisposing to nondisjunction (Henderson and Edwards 1968).
- Ova initially selected for ovulation are more likely to be normal than those remaining in older women i.e. production line hypothesis.
- Fertilization involving an aging oocyte (Simpson 1978; Martin-DeLeon and Williams 1987; Simpson and Golbus 1992).
- Certain parental metabolic derangements like thyroid disorders also influence the incidence of aneuploidy (Fialkow et al. 1965).

The chromosomal analysis of parents of the affected individuals are almost always normal. Occasionally parental mosaicism (46/47 + 21) (Weinstein and Warkany 1963; Kaffe et al. 1974) or another aberration may be detected. In such instances risk is presumed to be higher than for the appropriate parental age (Simpson and Golbus 1992).

In our study Chromosomal Studies of Parents having Down Syndrome children in 13 couples revealed chromosomal variants in 23% (6 of 26 individuals), which was not involving chromosome 21. Chromosomal variants may predispose to nondisjunction in Down syndrome because of interchromosomal effect (Farag et al. 1987; Martin et al. 1990). Inheritance of parental chromosomal variant in 3 Down syndrome cases out of the 6 chromosomal variant parents suggests an interchromosomal effect in them.

Translocation

In the present study only 4 (1.87%) cases had Down syndrome resulting from Robertsonian translocation.

The translocations most commonly involve a D group chromosome (nos. 13, 14 & 15) (Hecht et al. 1968). In the 4 cases with translocation, 2 cases had chromosome 21 translocated on D group chromosome i.e. chromosome 14 and 2 cases had translocation t(13;21), which is also a D/G translocation. With translocation D/G Down syndrome, one parent may have the same translocation chromosome in approximately 45% of such cases (Giraud and Mattei 1975). Three of the 4 cases of translocation of the present study had chromosomally normal parents. They are thus sporadic in origin having no parental association.

Maternal age also relates to the type of chromosomal abnormality. Translocation occurs 10% of the time in children born to mothers between 15 and 19 years of age (Rogers et al. 2000). In our study 25% (1out of 4) translocation was seen between 18 and 20 years of maternal age, and 75% between 21-34 age group (Group-II). Whenever chromosome analysis reveals a translocation, both parents should undergo karyotype study to check for a balanced translocation; however none of the 4 cases of translocation had balanced translocation in parents

Mosaicism

Mosaic (46/47, +21) is detected in 2% to 3% in Down syndrome (Chitham and MacIver 1965; Sutherland and Weiner 1972). The present study probably being on institutionalized population having a preponderence of Down syndrome had demonstrated a higher incidence (8.75%) 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 maternal age less than 35 years observed in 79% cases (Groups I & II) , really poses the question whether only advanced

maternal age should be considered as criteria for selecting women for pregnancy monitoring or should Down syndrome pregnancy screening be done routinely for all pregnant women. The mean maternal age was raised in free trisomy 21, but not in translocations (Mutton et al. 1996), 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.

CONCLUSION

The prenatal screening using maternal serum markers along with careful ultrasound scan in the second trimester to detect fetal neural tube defects, aneuploidies, other open structural defects, fetal loss and suboptimal pregnancy outcomes is well integrated in the health programs in the developed countries (Sweeney and Chescheir 1996). This prevention strategy has demonstrated reduction in the birth of Down syndrome babies. However, the value of routine pregnancy screening test for diagnosis of an abnormal fetus in the developing countries with scarce resources and skilled ultrasonographers is yet to be established. Until now there is neither a well accepted screening strategy for abnormal reproductive outcomes nor a large database on the Indian population. A community-based antenatal screening program would prove cost effective, if included in the Government Health Policy.

This study has provided the basis for further epidemiological surveys of Down syndrome in India in order to prepare the ground for an effective antenatal screening programme. Monitoring of all pregnancies for chromosomal disorders is the need of today as a preventive measure.

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REFERENCES

Brown MG, Lawce HJ 1997. Peripheral Blood Cytogenetic Methods. In: MJ Barch, T Knutsen and JL Spurbeck (Eds): *The AGT Cytogenetics Laboratory Manual,* 3rd Ed, Chap. 3, pp 77.

Chitham RG, MacIver E 1965. A cytogenetic statistical survey of 105 cases of mongolism. *Ann Hum Genet,* **28:** 309.

Down JLH 1866. Observations on an ethnic classification of idiots. *Clinical Lecture Reports, London Hospital,* **3:** 259.

Erickson JD 1978. Down syndrome, paternal age, maternal age and birth order. *Ann Hum Genet,* **41:** 289.

Farag TI, Krishna Murthy DS, Al –Awadi SA, Sundareshan TS, Ai-othman SA et al. 1987. Robertsonian translocation t dic (14p-22p) with regular trisomy 21 : a possible interchromosomal effect. *Ann Genet,* **30 (3):** 189.

Fialkow PJ, Uchida IA, Hecht F et al. 1965. Increased frequency of thyroid autoantibodies in mothers of patients with Down syndrome. *Lancet,* **2:** 868.

Giraud F, Mattei JF 1975. Aspects epidemiologiques de la trisome 21. *J Human Genetic,* **23:** 1.

Hecht F, Case MP, Lourien E et al. 1968. Nonrandomness of translocations in man. *Science,* **161:** 371.

Henderson SA, Edwards RG 1968. Chiasma frequency and maternal age in mammals. *Nature,* **218:** 22.

Holmes LB 1978. Genetic counselling for the older pregnant women : new data and questions. *N Engl J Med,* **28:** 1419.

Hook EG 1982. Epidemiology of Down Syndrome. In: SM Pueschel, JE Rynders: *Down Syndrome. Advances in Biomedicine and the Behavioral Sciences.* Cambridge Ware Press. pp 11.

Hook EB, Cross PK, Schreinemachers DM 1983. Chromosome abnormality rates in amniocentesis and in liveborn infants. *JAMA,* **249:** 2043.

Isaac GS, Krishnamurthy PS, Reddy YR, Ahuja YR 1985. Down Syndrome in Hyderabad, India. *Acta Anthropogenet,* **9 (4):** 256-60.

Kaffe S, Hsu LYDF, Hirschhorn K 1974. Trisomy 21 mosaicism in a woman with two children with trisomy 21 Down's syndrome. *J Med Genet,* **11:** 378.

Kaur M, Verma IC 1995. Serum Alpha Feto-Protein Screening in High Risk Pregnancies. *Indian J Pediatric,* **62:** 101.

Lejeune J, Gautier M, Turpin R 1959. Etude des chromosomes somatiques de neuf enfants mongoleins. *C R Acad Sci Paris,* **248:** 1721.

Martin RH, McGillivray B, Barclay L, Hildebrand K, Ko E 1990. Sperm chromosome analysis in a man heterozygous for a reciprocal translocation 46,XY t(12;20) (q24.3;q11). *Hum Reprod,* **5 (5):** 606.

Martin-De Leon PA, Williams MB 1987. Sexual behaviour and Down syndrome; the biological mechanism. *Am J Med Genet,* **27:** 693.

McLaren J, Bryson S 1987. Review of recent epidemiological studies of mental retardation : Prevalence, associated disorders and etiology. *Am J Mental Retardation,* **92:** 243-254.

Mutton D, Alberman E, Hook EB 1996. Cytogenetic and epidemiological findings in Down syndrome, England and Wales 1989 to 1993. National Down Syndrome Cytogenetic Register and the Association of Clinical Cytogeneticists. *J Med Genet,* **33 (5):** 387-94.

Penrose 1933. The relative effects of paternal and maternal age in mongolism. *J Genet,* **27:** 219.

Rogers PT, Roizen NJ, Capone GT 2000. Down Syndrome. In : AJ Capate, PJ Accardo (Eds): *The Spectrum of Developmental Disabilities.* 2nd Ed,

Vol. II, Chap. 15, pp 221-241.
Simpson JL, Golbus MS 1992. Genetic of Pregnancy Losses. In: *Genetics in Obstetrics and Gynecology.* 2nd Ed. Chap 14, pp 181-200. W.B. Saunders Company.
Simpson JL 1978. Genetic consequences of aging sperm or aging ova : Animal studies and relevance to humans. In : JJ Sciarra, GE Zatuchnni, JJ Speidel (Eds): *Risks, Benefits and Controversies in Fertility Control*. New York, Harper and Row, p 506.
Sutherland GR, Wiener S 1972. Cytogenetics of 271 mongols. *Aust Paediatr*, **8:** 90.
Sweeney WJ, Chescheir NC 1996. Evaluations of elevated Maternal serum α-fetoprotein. In Prenatal Diagnosis and Reproductive Genetics, JA Kuller, NC Chescheir, RC Cefalo (Eds.) Chap11. pp 79.
Sumner AT 1982. The nature and mechanisms of chromosome banding. *Cancer Genet Cytogenet*, **6:** 59.
Verma IC, Mathew S, Elango R, Shukla A 1991. Cytogenetic studies in Down syndrome. *Indian Pediatr*, **28:** 991.
Waardenburg PJ 1932. *Das menschliche Auge und seine Erbanlagen*.The Hague: Nijhoff.
Weinstein ED, Warkany J 1963. Maternal mosaicism and Down Syndrome. *J Pediatr*, **63:** 599.
Yunis J, Ball D,Sawyer J 1979. G-banding patterns of high resolution human chromosomes 6-22, X and Y. *Hum Genet*, **49:** 291-306.