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Cytogenetic Findings and Risk Factors for Down Syndrome in Punjab

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ABSTRACT The aim of the present study was to analyse karyotypic pattern, clinical features and factors responsible for the risk of Down syndrome (DS). Chromosomal investigations were done on 114 cases of Down syndrome referred to the Department of Human Genetics, Guru Nanak Dev University, Amritsar. Among 114 cases, 100(87.71%) showed free trisomy 21, mosaicism was present in 6(5.26%) cases and translocations were seen in 3(2.63%) cases. A case of double aneulpoidy was also seen. Average maternal age at the time of birth of DS child was 27.5 years. We found that 25.44% mothers experienced one miscarriage before the birth of a DS child and 7.89% had death/still birth. Average age of DS child referred to the Department was 3.5 years (44 months 6 days) and most of them were either first or second born. In the present study, 49.12% of DS children were diagnosed within first year of life. About 14.9% parents of DS were daily wagers. Birth of DS children was higher in the month of February and least in the month of October. Early diagnosis, karyotyping and awareness about screening tests can prove helpful in decreasing genetic burden.

INTRODUCTION

Down syndrome is a common genetic condition for referral to genetic clinics and is the most frequent live born aneuploidy affecting 4% of all clinically recognized human pregnancies (Reevees et al. 2001). The incidence of DS in India is around 1 in 1200 at the age of 25 years (Patel and Adhia 2005). It is associated with mental retardation, cognitive impairment, developmental delay, heart defects, leukemia, Alzheimer's disease, immunological impairments etc. (Lott and Head 2005; Ram and Chinen 2011). Though advanced maternal age has been identified as a risk factor for DS, however, number of younger mothers bearing DS children has also increased. The reason for nondisjunction is not clearly understood. It has been suggested that risk for DS may be accelerated by various environmental factors that attributed to genetic polymorphism in folate metabolism pathway. The present study aimed to evaluate the karyotype, clinical history, detailed pedigree analysis and possible risk factors for having DS child in Punjab.

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MATERIAL AND METHODS

Cytogenetic investigations were carried out on 114 DS cases referred during 2007-2012 to the Department of Human Genetics, Guru Nanak Dev University, Amritsar, from different areas of Punjab. This study was approved by the ethics review board of Guru Nanak Dev University. A standard questionnaire was prepared to record detailed personal information, family history and clinical investigations after taking the informed consent from the parents. Peripheral blood lymphocytes of DS children were cultured using standard protocol with modifications (Kaur et al. 2003). After banding 50 metaphases were scanned for each case on Olympus BX51 microscope and 10 metaphases were analysed by automated karyotyping system (Cytovision, Applied Imaging).

RESULTS

The chromosomal investigations were undertaken in 114 cases, out of which 100(87.71%) cases showed free trisomy, 6(5.26%) and 3(2.63%) cases showed mosaicism and translocation, respectively. Among these cases, 4 children had features of DS and showed normal karyotypes whereas one case showed double aneuploidy. Translocation t(21q;21q) was seen in two males and one female child (Table 1). Mean maternal age in the present study was 27.5 years, mean paternal age was 31.3 years and

maternal grandmother's age was 52.6 years (Table 2). The clinical features observed in the present study were epicanthal folds, protruding tongue, depressed nasal bridge, chest infections, sleep apnea etc. (Table 3). In the present study, 25.44% mothers experienced one spontaneous miscarriage, 7.89% had it twice, 1.7% experienced it thrice and 7.89% cases showed death/ still births before the birth of DS child (Table 4). The family investigations enabled us to determine the order of DS child among the sibship (Table 5). A higher number of DS children were of 1st and 2nd order, born to younger mothers. In the present study, 49.12% DS children were referred within 1st year of age, 40.4% within 10 years, while 10.52% were referred till the age of 20 years. We observed that 33.3% parents of DS children were engaged in jobs, 37.7% were having their own business, 14.03% were involved in agriculture and 14.9% parents were daily wagers. Among the parents in the study, 73(64.03%) were illiterate or studied upto high school level and 41(35.96%) were graduates/post graduates and 15(13.15%) of the latter were from rural background. It was interesting to observe that 18.4% of DS children were born in the month of November while minimum of 3.51% in the month of July (Table 6).

Table 1: Cytogenetic profile of 114 cases of Down syndrome

Karyotype	*Male*	*Female*	*Combined*
Free trisomy 21	62 (54.38%)	38 (33.33%)	100 (87.71%)
Mosaicisim	3 (2.63%)	3 (2.63%)	6 (5.26%)
Robertsonian translocation			
47,XY,t(21:21)	2 (1.75%)	-	3 (2.63%)
47,XX,t(21q:21q)/ 46,XX	-	1 (0.88%)	
Others			
48,XXY,+21	1 (0.88%)	-	1 (0.88%)
46,XX; 46, XY	2 (1.75%)	2 (1.75%)	4 (3.51%)

DISCUSSION

Trisomy 21 is a common birth defect and can be diagnosed easily on the basis of clinical fea-

Table 2: Mean maternal, paternal and maternal grandmother ages at the time of birth of DS child

Ages (mean in years)	*Mean age*	*Trisomy 21*	*Translocation*	*Mosaics*	*Double aneuploidy (48,XXY,+21)*	*Others*
Maternal	27.5	27.45	30	26.8	31	28.25
Paternal	31.3	31.23	32	32.2	31	31.5
Grand mother	52.6	52.45	53.3	53	55	53.5

Table 3: Comparison of clinical features and physical abnormalities in DS.

Features	*Present study (%)*	*Azman et al. 2007*	*Kava et al. 2004*	*Kumar et al. 2001*	*Jones et al. 1997*	*Fryns et al. 1990*
Simian crease	45.61%	36.8%	33.2%	40%	45%	48%
Epicanthal folds	78.1%	17.5%	56.9%	60%	-	40%
Gap b/w 1st and 2nd toe	45.01%	33.3%	46.2%	-	-	45%
Protruding tongue	67.0%	19.2%	29.9%	-	-	-
Depressed nasal bridge	57.01%					
High arched palate	16.66%					
Short broad hands, incurved finger	37.71%	24.5%, 19.2%	36.1%	50%	50%	62%
Chest infection	55.26%					
Constipation	28.07%					
Developmental Delay	59.0%					
Palpebral fissures	39.47%	89.3%	83.9%	-	80%	80%
Low set, small ears	54.38%	56.1%	66.9%	-	60%	50%
Sleep apnea	21.92%					
Jaundice (within 48 hrs of birth)	26.31%					
Respiratory distress	9.64%					
Liver enlarged (congenital)	0.87%					
leukemia (congenital)	1.75%					
Heart problems/ hole in heart	12.3%					
Neonatal asphyxia	5.26%					
Anal blockage(congenital)	1.75%					
Under developed genitalia	1.75%					

Table 4: Frequency of miscarriages in relation to age of mother of DS

Age range (mothers)	*Number of miscarriages*			*Death /still births*	*Total*
	1	*2*	*3*		
20-30 (group I)	16 (14.03%)	4 (3.5%)	1 (0.87%)	5 (4.38%)	26 (22.8%)
31-40 (group II)	8 (7.01%)	3 (2.6%)	1 (0.87%)	2 (1.75%)	14 (12.3%)
41-50 (group III)	5 (4.38%)	2 (1.75%)	-	2 (1.75%)	9 (7.9%)
Total	29 (25.44%)	9 (7.89%)	2 (1.7%)	9 (7.89%)	49 (42.9%)

Table 5: Distribution of DS children according to their order.in sibship

Age range(mothers)	*Order in sibship*						
	1	*2*	*3*	*4*	*5*	*6*	*7*
20-30(Group I)	46 (40.3%)	19 (16.6%)	5(4.38%)	1(0.87%)	-	-	-
31-40(Group II)	7 (6.14%)	12 (10.5%)	7 (6.145)	2 (1.7%)	1 (0.87%)	-	1 (0.87%)
41-50(Group III)	2 (1.7%)	3 (2.6%)	5 (4.38%)	-	2 (1.7%)	1 (0.87%)	-
Total	55 (48.2)	34 (29.8%)	17 (14.9%)	3 (2.65)	3 (2.6%)	1 (0.87%)	1 (0.87%)

Table 6: Distribution of DS cases according to their month of conception and Birth

Months	*Number of conception*	*Number of birth*
January	12 (10.5%)	16 (14.03%)
February	21 (18.4%)	05 (4.4%)
March	13 (11.4%)	08 (7.02%)
April	16 (14.03%)	06 (5.26%)
May	05 (4.4%)	07 (6.14%)
June	08 (7.02%)	10 (8.8%)
July	06 (5.265)	04 (3.51%)
August	07 (6.14%)	07 (6.14%)
September	10 (8.8%)	05 (4.4%)
October	04 (3.51%)	12 (10.5%)
November	07 (6.14%)	21 (18.4%)
December	05 (4.4%)	13 (11.4%)

tures. However, karyotyping is necessary for the confirmation of free trisomy 21, mosaicism and translocation in DS children for determining the recurrent risk and to provide genetic counseling. In the current study the frequency of non-disjunction, mosaicism and translocation was 87.71%, 5.26% and 2.63% respectively. Our result (87.71%) was comparable to other Indian studies which shows a wide range of the non-disjunction frequency (80-97%). The frequency of non-disjunction among international studies ranges from 92.2-96.9%, while only study from Jordan revealed a lower frequency of 85%. A 2 years old male was referred to the Department for chromosomal analysis, he exhibited double aneuploidy, 48,XXY,+21 and showed features of DS. Double aneuploidy occurs due to double events of non-disjunction resulting in single abnormal gamete or separate events during gametogenesis in both parents (Ford et al. 1959). Various studies have reported the frequency of non-classical type of DS to be 0.4 -2.4% whereas in our study it was 0.88% (Table 7). The normal karyotype seen in four children will be further investigated using FISH.

The risk of having a child with DS increases with the advanced maternal age (Connor et al. 1991; ACOG 2007). However, other environmental factors are also known to be involved such as paternal age, nicotism, infections, irradiations, hormonal imbalances, regional and seasonal variation. A study on Malaysian women reported that 64% of mothers were older than 35 years of age and 36% of mothers had average age of 32.3 years (Azman et al. 2007). The study on Moroccan mothers revealed that their mean maternal age was 35.39 years (Jaouad et al. 2010). Indian studies have reported that 75%-82% of DS children were born to mothers younger than 30 years (Kaur and Verma 1995; Jyothy et al. 2000; 2001; Kothare et al. 2002; Malini and Ramachandra 2006; Kaur and Singh 2010). In our study, mean maternal age at non-disjunction (27.5 years) is slightly less as compared to other studies. This could be due to regional, nutritional and seasonal variations. The mechanism behind the non-disjunction is not well understood. One of the reasons could be that the ovaries of young mothers are biologically older than their chronological age which may lead to increased incidence of non-disjunction (Schupf et al. 1994). A few reports indicate the influence of grand maternal age on the risk of their grand-child being born with DS (Nazmi and Suhair 2010; Malini and Ramchandra

Table 7: Comparative cytogenetic studies

Study	*Number*	*Trisomy 21*	*Mosaics*	*Translocation*	*Non-classical*
Poddar et al. (2012) India	45	93.33%	6.7%		
Qahatani et al. (2011) Jeddah	72	94.4%	1.5%	4.1%	
El-Gilany et al. (2011) Egypt	712	96.1%	0.8%	2.7%	3.1%
Kanwar et al. (2010) Jordan	33	85.0%	6.0%	1.0%	
Jaouad et al. (2010) Morocco	852	96.2%	0.59%	3.17%	
Chandra et al. (2010) India	1020	83.82%	10.78%	5.0%	0.4%
Jayalashamma et al. (2010) India	874	86.9%	4.3%	8.8%	
Bisseli et al. (2009) Brazil	387	92.2%	1.5%	1.5%	
Azman et al. (2007) Malaysia	149	94.6%	4.7%	0.7%	
Malini and Ramchandra (2007) India	150	81.33%	1.33%	0.67%	
Sheth et al. (2007) India	382	84.28%	3.9%	8.9%	2.4%
Mokhtar et al. (2003) Egypt	673	95.4%	0.7%	2.7%	1.2%
Jyothi et al. (2000) India	1001	87.92%	7.69%	4.39%	
Thomas et al. (1992) India	316	86.6%	5.8%	7.7%	
Verma et al. (1991) India	645	93.00%	2.6%	4.10%	
Stoll et al. (1990) France	391	94.1%	2.3%	3.6%	
Al-Awadi et al. (1990) Kuwait	635	96.2%	1.4%	1.9%	0.65
English et al. (1989) England	65	96.9%	1.5%	1.5%	
Ambani et al. (1984) India	146	83.60%	9.60%	6.80%	
Murthy et al. (1981) India	113	80.53%	10.62%	8.80%	
Mulcahy et al. (1979) Australia	222	95.0%	2.0%	5.33%	
Verma et al. (1979) India	150	92.0%	2.0%	5.33%	
Rafi and Marimuthu (1977) India	92	97.83%		2.17%	
Phadke et al. (1975) India	136	97.77%		2.20%	
Present study	114	87.71%	5.26%	2.63%	0.88%

2011). At an advanced age, the grandmother's reproductive system fails to make the essential proteins needed for proper meiotic segregation in the germ cell of her daughter, leading to non-disjunction of chromosome 21 during the embryogenesis of DS child's mother when she was in the grand mother's womb (Malini and Ramachandra 2006); on the contrary other reports fail to support the same (Allen et al. 2009; Kovaleva et al. 2010). The present study observed that mean maternal grandmother's age at the time of birth of DS child was 52.6 years in case of free trisomy, 53.3 years in translocation and 53 years in mosaics (Table 2). The age of grandmother was recorded at the time of birth of DS child. A much higher association has been observed between the age of mother and grand maternal age (r- 0.000, $p< 0.001$).

Among the clinical features, epicanthal folds (78.1%) were most commonly observed by us followed by protruding tongue (67%), developmental delay (59%), depressed nasal bridge (57.01%), chest infection (55.26%), low set and small ears (54.38%), simian crease (45.61%), palpebral fissures (39.47%), sleep apnea in 21.92% cases, respiratory distress in 9.64% cases and the CAD problems were present in 12.3% cases. Some studies have reported upslanting palpebral fissure as the most commonly observed feature (Table 3). Further, we observed that 26.31% of DS children had jaundice within 48 hrs of birth and this has not been reported earlier.

We observed that 25.44% mothers had at least one miscarriage and 7.89% had death/still birth and among these 22.8% were below the age of 30 years (Table 4). Similar studies suggest that relative risk to produce a DS child may be associated with increased number of abortions in younger women (Schupf et al. 1994; Rajangam et al. 1997; Kothare et al. 2002; Poddar et al. 2012). Our data suggests that younger mothers (<35 years) having higher number of miscarriages are at higher risk of having child with DS.

Most of the DS families coming to us had two children and the birth order of DS child was either 1st or 2nd. Only 5 families had 5th, 6th, and 7th order of DS child (Table 5). On the contrary, a study in UAE reported that a child with DS was mostly last or second last child (Murthy et al. 2007). Some reports suggest that risk of DS increases with increased parity in both young and old mothers (Cutler et al. 1986; Doria–Rose et al. 2003) while Chan et al. (1998) contradict this observation. The age of presentation of Down syndrome cases referred to us ranged from 2 days to 20 years. Average age of presentation

was 3.5 years (44 months 6 days) and all the cases were diagnosed postnatally. It has been reported that there is link between education of parents and use of health services (Khoshnood et al. 2004; Dzurova and Pikahart 2005; Poddar et al. 2012). We also observed a positive correlation between higher socioeconomic status of parents and recognition age of DS children (r-0.030, $p < 0.005$). We observed that though most of the parents in the study were educated but lacked awareness about Down syndrome or any other screening tests available.

A strong preference for male child has been observed among Punjabi society and has led to the declining sex ratio. People adopt various methods to get a son and use Sex Selection Drugs which are consumed in first trimester usually, a critical period for the fetal development. In the present study, 25.44% mothers took such drugs for having male child. These drugs put the new born to four times higher risk of congenital malformations and could be a factor for non-disjunction in these women (Bandoypadhyay and Singh 2007). Seasonal/monthly variation were also noticed by us. DS births were highest in the month of November and least in the month of July (Table 6). Studies indicate that defect in the folic acid metabolism due to mutations in *MTHFR, MTR, MTRR* and *RFC 1* may be a possible cause of chromosomal non-disjunction.

Chromosomal analysis is important in Down syndrome children to see if the child has free trisomy, translocation or mosaicism. Preconceptional folic acid supplementation should be recommended to women of reproductive age. Parents should be discouraged to use sex selection drugs. Information about DS and awareness about genetic disorder should be given to the parents and young couples and it will be helpful in preventing it. The early intervention like prenatal screening methods, biochemical, ultrasound screening, amniocentesis and chrionic villus sampling combined with karyotyping and genetic counseling can decrease genetic burden.

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