Cytogenetic and clinical profile of Down syndrome in Northeast Malaysia

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ABSTRACT

Introduction: This study was designed to evaluate the karyotype pattern, clinical features and other systemic anomalies of patients with Down syndrome in Malaysia.

Methods: Retrospective analysis was performed on the case records of 149 patients confirmed as Down syndrome by cytogenetic analysis at Human Genome Centre and Genetic Clinic at the Universiti Sains Malaysia.

Results: Among the 149 cases of Down syndrome presenting over a period of 4.2 years, free trisomy (non-disjunction) was present in 141 cases (94.6 percent). One case (0.7 percent) had translocation, and seven cases (4.7 percent) were mosaics. Average age at presentation was 10.6 months. Average maternal age at birth of the affected child was 32.3 years. The prominent craniofacial features noted were upslanting palpebral fissures (89.3 percent), flat facial profile (64.9 percent), low set ears (56.1 percent), epicanthic folds (17.5 percent) and protruding tongue (19.2 percent). A total of 52.6 percent of the cases had documented hypotonia. Characteristic limb and dermatoglyphic anomalies included short stubby fingers (24.5 percent), sandal gap (33.3 percent), unilateral or bilateral simian crease (36.8 percent) and clinodactyly (19.2 percent). Ophthalmological abnormalities, such as hypertelorism, were presented in 33.3 percent of the cases. Congenital heart disease was diagnosed in 35 out of 71 cases (49.3 percent) and gastrointestinal anomalies were noted in 18 out of 79 cases (22.7 percent) analysed.

Conclusion: Efforts to establish early diagnosis and a proper screening for high association with systemic anomalies should be undertaken among the Down syndrome patients in this population.

Keywords: cytogenetic analysis, Down syndrome, karyotype pattern, non-disjunction, trisomy 21

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INTRODUCTION

Down syndrome is the most common chromosomal aneuploidy, with the incidence ranging from one in 600 to one in 1,000 live births.[1] Down syndrome results from the presence of an extra chromosome 21, either as trisomy or as part of Robertsonian translocation. In most cases of Down syndrome, the extra chromosome is present as a result of a chromosomal failure to segregate during meiosis. Despite the fact that Down syndrome has a particular combination of phenotypic features that include mental retardation and characteristic facies, clinical diagnosis of Down syndrome may be non-confirmatory in one-third of the cases.[2] Every patient suspected of having Down syndrome should undergo cytogenetic analysis because karyotyping is essential for conformation of the diagnosis, determination of recurrence risk and for genetic counselling. The aim of this study was to analyse the karyotype pattern, clinical features and other systemic anomalies of patients with Down syndrome in our cohort of patients and to correlate the findings with maternal age.

METHODS

The Human Genome Centre (HGC), Universiti Sains Malaysia (USM), is a referral laboratory for cytogenetic investigation for the whole of Malaysia. Out of several cases referred to the HGC, USM during the period from January 2001 to March 2006, 149 patients with a cytogenetically-confirmed diagnosis of Down syndrome were included in this study. Cytogenetic analysis was carried out at HGC, employing microculture of the peripheral blood lymphocytes of the study subjects at 37 °C for 72 hours, and cultures were harvested by standard cytogenetic procedures. GTG-banded chromosomes were karyotyped according to International System for Human Cytogenetic Nomenclature (ISCN, 1995)[3] and the abnormalities were detected. Retrospective analysis of clinical features and other associated systemic anomalies were performed on these patients based on data obtained

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from their case records. Even though karyotype analysis was performed on all 149 cases, the correlation with maternal age could only be looked into in 144 case, since in five cases, the maternal age was not stated. The age of presentation could only be traced in 105 cases because it was not properly documented for the other 44 cases. Clinical features (craniofacial, limb and dermatoglyphic anomalies) were available only in 57 cases and these were included in the study of the clinical profile of Down syndrome. Details of heart defects were available for 71 cases. Details of association with anomalies of gastrointestinal system and hypothyroidism were available for 79 cases.

RESULTS

All 149 cases (70 males and 79 females; male to female ratio, 1:1.1) included in this study were cytogenetically-confirmed cases with a clinical diagnosis of Down syndrome. Out of these, 141 cases (94.6%) were found to have non-disjunction trisomy 21, one case had translocation (0.7%) and seven cases (4.7%) were mosaic trisomy 21. With regard to maternal age, 64% of the mothers were older than 35 years of age, while the remaining 36% of mothers were less than 35 years of age at the time of birth of the affected child. Average maternal age at birth of the affected child was 32.3 (range 21–50) years, and the distribution of cases with respect to the maternal age at birth is presented in Table I. The average age at presentation was 10.6 months (range 2 days–12 years). Males and females were affected almost in the same ratio (1: 1.1); no significant difference was noticed, even in the younger maternal age groups, except for the mothers in the less than 25 years age group, where females were more affected than males. However, the number of

Table I. Correlation of maternal age and chromosomal aberration in Down syndrome.

Maternal age (years)	n = 144	Ratio (male:female)	Cytogenetic profile	No. of cases	Total percentage (%)
Group I	8	1 : 7	Trisomy 21	8	
(≤ 25)			Translocation	–	5.6
			Mosaic	–	
Group II	14	1.3 : 1	Trisomy 21	13	
(26–30)			Translocation	1	9.7
			Mosaic	–	
Group III	27	1 : 1.2	Trisomy 21	24	
(31–35)			Translocation	–	18.8
			Mosaic	3	
Group IV	43	1 : 1	Trisomy 21	42	
(36–40)			Translocation	–	29.9
			Mosaic	1	
Group V	52	1 : 1.1	Trisomy 21	50	
(> 40)			Translocation	–	36.1
			Mosaic	2	

Table II. Comparison of dysmorphic features and physical abnomalities in Down syndrome.

Dysmorphic features	Current study (%)	Kava et al[14] (%)	Kumar et al[21] (%)	Jones[22] (%)	Fryns[23] (%)
Upslanting palpebral fissures	89.3	83.9	–	80	80
Flat facial profile	64.9	50.9	–	90	90
Ears abnomality	56.1	66.9	–	60	50
Hypotonia	52.6	76.3	80	80	21–77
Simian crease	36.8	33.2	40	45	48
Sandle sign	33.3	46.2	–	–	45
Hypertelorism	33.3	33.9	–	–	–
Short stubby fingers	24.5	–	–	–	–
Protruding tounge	19.2	29.9	–	–	–
Clinodactyly	19.2	36.1	50	50	62
Epicanthic folds	17.5	56.9	60	–	40
Excessive skin fold on neck	12.2	36.8	–	80	81/85

samples is too small for a firm conclusion (Table I).

Among the clinical features listed in Table II, it can be seen that craniofacial abnormalities comprised upslanting palpebral fissures (89.3%), flat facial profile (64.9%), low set ears (56.1%), epicanthic folds (17.5%) and protruding tongue (19.2%). A total of 52.6% cases had documented hypotonia. Characteristic limb and dermatoglyphic anomalies were seen in less than one-half of cases. These included short stubby fingers (24.5%), sandal gap (33.3%), unilateral or bilateral simian crease (36.8%), and clinodactyly (19.2%). Ophthalmological abnormalities, such as hypertelorism, were observed in 19 cases (33.3%). Congenital heart disease was diagnosed in 35 out of 71 cases screened for heart defects. The cardiac anomalies were patent foramen ovale (22.8%), ventricular septal defect (20%), atrioventricular septal defect (20%), atrial septal defect (17.1%), patent ductus arteriosus (11.4 %) and tetralogy of Fallot (8.5%). Gastrointestinal anomalies were present in 18 out of 79 cases (22.7%). Imperforated anus was seen in four cases (22.2%), and there were three cases (16.6%) of Hirschsprung's disease and one case (5.5%) each with anorectal malformation and morgagni hernia. Hypothyroidism was encountered in seven cases (8.9%) and one case (1.3%) presented with acute leukaemia.

DISCUSSION

The clinical diagnosis of Down syndrome usually presents with no particular difficulty. The diagnostic accuracy of Down syndrome on the basis of clinical features in the neonatal period has been reported to range from 73% to 100%.[4,5] Nevertheless, even an experienced physician may find it occasionally difficult to give a confirmatory diagnosis on an infant when the clinical features may be minimal. Karyotyping is essential for confirmation of the clinical diagnosis, determination of recurrent risk and to provide a basis for genetic counselling. Although the particular karyotype responsible for Down syndrome has little, if any, effect on the phenotype of the patient, it is essential for determining the risk of recurrence. In an earlier report, the incidence of Down syndrome in Malaysia has been reported as one in 950 and little variation has been reported among the three largest ethnic groups (Malay 1:981, Chinese 1:940, Indians 1:860).[6] Unfortunately, there are no reports on the recent incidence of Down syndrome in the Malaysian population.

The frequencies of the different karyotype patterns observed in these subjects are shown in Table I. The percentage of free trisomy 21 was 94.6 %, translocation trisomy 0.7% and mosaic trisomy 4.7%. The frequency of mosaic trisomy was higher than that of translocation among our Down syndrome patients. This data is relatively compatible with the data from a few international studies (Table III)[7-11] and slightly deviating from a few other studies.[12-14] Thomas et al from Bangalore, India reported a higher frequency of mosaicism with 86.6% free trisomy, 7.7% translocation and 5.8% mosaicism.[12] Similarly, Jyothy et al from India reported a high prevalence of mosaicism (7.7%).[13] Contrary to this, Mutton et al from England and Wales reported trisomy in 95%, translocation in 4% and mosaicism in 1% children with Down syndrome.[10] Correspondingly, Kava et al found free trisomy in 95%, translocation in 3.2% while 1.8% were mosaics.[14] Even though no specific reason could explain this discrepancy in the frequency of karyotype pattern in Down syndrome patients, differences in time period, the maternal age and population studied could be contributing factors. For non-disjunction trisomy 21, the most common error is maternal non-disjunction in the first meiotic division, with meiosis I error occurring three times as frequently as meiosis II errors. Most mosaic cases result from a trisomic zygote with mitotic loss of chromosome 21. The Down syndrome cases with unbalanced translocation usually are *de novo* and nearly 25% result from familial transmission. The one case of translocation observed in the present study was the result of a Robertsonian translocation between chromosome 14 and 21, and had arisen *de novo*. Both parents of this translocation Down syndrome child showed normal karyotype. Advanced maternal age remains the only well-documented risk factor for maternal meiotic nondisjunction.

Table III. Frequencies of different karyotypes among the studied Down syndrome cases and pooled data from worldwide surveys.

Source	Total no.	Regular trisomy		Translocation		Mosaic		Non-classical	
		No.	%	No.	%	No.	%	No.	%
Malaysia (Current study)	149	141	94.6	1	0.7	7	4.7	–	–
Scotland[7]	153	144	94.1	2	1.3	7	4.6	–	–
France[8]	391	368	94.1	14	3.6	9	2.3	–	–
Egypt[9]	673	642	95.4	18	2.7	5	0.7	8	1.2
England and Wales[10]	5,737	5,411	94.3	220	3.8	66	1.2	40	0.7
Belgium[11]	88	81	92.1	6	6.8	1	1.1	–	–

In our study population, average maternal age at birth of the affected child was 32.3 years. Out of the 149 Down syndrome patients, 64% were born to mothers older than 35 years of age. This clearly indicated that maternal age was a major contributing risk factor in a significant proportion of cases in this population (Table I). Almost a double increment was noted in the percentage of cases between the group of young mothers (Group I, Group II and Group III) and the difference was reduced as the maternal age approaches 40 years (Table I). This data is consistent with the exponential increment noted by Epstein.[15] It is well known that aneuploidy can have major detrimental health consequences when it occurs in either germinal or somatic cells. Germinal aneuploidies, a major cause of pregnancy loss, aneuploid births and developmental defects,[16] are thought to arise *de novo*, through meiotic errors in germ cells of either parents, or mitotically shortly after fertilisation. Both age-dependent and age-independent factors appear to be operating simultaneously. It could be due to age-dependent decay in the spindle fibres or their components, a failure in nucleolar breakdown or an accumulation of the effects of radiation, hormonal imbalances and infection.[17] On the other hand, clinical and experimental studies have shown that age-independent DNA hypomethylation is associated with chromosomal instability and abnormal segregation. Based on this, Christman et al suggested a link between dietary folate and methyl deficiency in vivo and DNA hypomethylation.[18] On the basis of these cellular observations, James et al and Hobbs et al have postulated a link between abnormal folate metabolism and mutation of the methylenetetrahydrofolate reductase gene, hence as a risk factor for nondisjunction and Down syndrome in younger (< 35 years) mothers.[19,20] Further research on the role of polymorphism of other genes involved in folate metabolism as a causative factor for maternal nondisjunction and high risk genetic factor for Down syndrome is warranted, in view of the high proportion of young mothers giving birth to Down syndrome children.

Craniofacial features were the foremost indicators of clinical suspicion of Down syndrome. Among the craniofacial features studied, upslanting palpebral fissures was the most frequent feature observed (89.3%), which is in agreement with other studies as shown in Table II. The major four clinical features present in more than 50% of the cases were the upslanting palpebral fissures, flat facial profile, ear abnormality and hypotonia. The observation of simian crease, sandal gap and hypertelorism in more than 30% of the total cases in the present study are consistent with a study by Kava et al.[14] However, with regard to the other clinical features, inconsistencies were noted when compared to the other studies (Table II).[21-23] The incidence of heart defect was encountered in 49% of our study subjects. It was interesting to note that heart diseases were predominant among Down syndrome children born to mothers aged more than 35 years. The most common defect observed was patent foramen ovale (22%) followed by ventricular septal defect (20%), atrioventricular septal defect (20%) and atrial septal defect (17%). To the best of our knowledge, this is the first report on karyotype pattern, clinical features and maternal age associated with Down syndrome in Malaysia.

Despite continued work, no notable medical treatment for mental retardation associated with Down syndrome has been forthcoming. Early intervention programmes, which are individualised programmes designed to meet the specific needs of each Down syndrome patient, should be implemented at any time shortly after birth. Early intervention helps in each of the four main areas of development: gross motor and fine motor skills, language, social development and self-help skills. Taking this fact under consideration, the age of diagnosis and the age of presentation are important factors for the success in managing Down syndrome patients. In this study, the average age at presentation was 10.6 months (range 2 days–12 years). Only 41% of the cases were diagnosed during the neonatal period, while the other 59% were diagnosed at the age older than one month. This resulted in a delayed evaluation and postponed the implementation of early intervention programmes as well as appropriate therapy for conditions, such as cardiac disease, gastrointestinal anomalies and hypothyroidism in more than half of the cases. This obvious delay in the diagnosis of Down syndrome could be due to the lack of antenatal screening, a fairly large number of births taking place outside the hospital set-up, and lack of regular neonatal check-up by a trained neonatologist or paediatrician.

Every child with Down syndrome should have an access to an early intervention programme as soon as possible. Early intervention programmes may improve the patient's social quotients. Programmes for infants age ≤ 3 years should be designed to comprehensively monitor and enrich their development by focusing on feeding, as well as gross and fine motor skills, language, personal and social development. Many children and adults can enhance the quality of life through speech and physical therapies, regular medical check-ups and treatment, nutritional therapy and occupational therapy. Parents of Down syndrome children should be provided with referrals to support groups and organisations that advocate for persons with Down syndrome and their families. Improvement in medical care, early intervention, special education, and vocational counselling and training, will improve the quality of life for Down syndrome patients and increase their life expectancies. In conclusion, efforts to establish early diagnosis, and a proper screening for high association with systemic anomalies, should be undertaken among the Down syndrome patients in this population.

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REFERENCES

1. Smith G, Berg J. Down's Anomaly. 2nd ed. Edinburgh and New York: Churchill Livingstone, 1976.
2. Hindley D, Medakkar S. Diagnosis of Down's syndrome in neonates. Arch Dis Child Fetal Neonatal Ed 2002; 87: F220-1. Comment in: Arch Dis Child 2004; 89:691.
3. Mitelman F, ed. An International System for Human Cytogenetic Nomenclature (1995). Basel: S. Karger, 1995.
4. Fried K. A score based on eight signs in the diagnosis of Down syndrome in the newborn. J Ment Defic Res 1980; 24:181-5.
5. Devlin L, Morrison PJ. Accuracy of the clinical diagnosis of Down syndrome. Ulster Med J 2004; 73:4-12.
6. Hoe TS, Boo NY, Clyde MM. Incidence of Down's syndrome in a large Malaysian matemity hospital over an 18 month period. Singapore Med J 1989; 30:246-8.
7. Speed RM, Johnston AW, Evans HJ. Chromosome survey of total population of mentally subnormal in north-east of Scotland. J Med Genet 1976; 13:295-306.
8. Stoll C, Alembik Y, Dott B, Roth MP. Epidemiology of Down syndrome in 118,265 consecutive births. Am J Med Genet 1990; 7:79-83.
9. Mokhtar MM, Abd El Aziz AM, Nazmy NA, Mahrous HS. Cytogenetic profile of Down syndrome in Alexandria, Egypt. Eastern Mediterr Health J [online] 2003; 9: Nos 1/2. Available at: www.emro.who.int/publications/emhj/0901_2/cytogenetic.htm. Accessed August 15, 2006.
10. Mutton D, Alberman E, Hook EB. Cytogenetic and epidemiological findings in Down syndrome, England and Wales 1989 to 1993. J Med Genet 1996; 33:387-94. Comment in: J Med Genet 1996; 33:806.
11. Cassiman JJ, Fryns JP, De Roover J, Van den Berghe H. Sex chromatin and cytogenetic survey of 10417 adult males and 357 children institutionalized in Belgian institutions for mentally retarded patients. Humangenetik 1975; 28:43-8.
12. Thomas IM, Rajangam S, Hegde S. Cytogenetic investigations in Down syndrome patients and their parents. Indian J Med Res 1992; 96:366-71.
13. Jyothy A, Kumar KS, Rao GN, et al. Cytogenetic studies of 1001 Down syndrome cases from Andra Pradesh, India. Indian J Med Res 2000; 111:133-7.
14. Kava MP, Tullu MS, Muranjan MN, Girisha KM. Down syndrome: clinical profile from India. Arch Med Res 2004; 35:31-5. Comment in: Arch Med Res 2005; 36:183; author reply 184-5.
15. Epstein CJ. Down syndrome. In: Scriver CR, Beaudet AL, Sly WS, eds. The Metabolic and Molecular Bases of Inherited Disease. 7th ed. New York: McGraw Hill, 1995.
16. Wyrobek AJ, Marchetti F, Sloter E, Bishop J. Chromosomally defective sperm and their developmental consequences. In: Anderson D, Karakaya AE, Sram RJ, eds. Human Monitoring after Environmental and Occupational Exposure to Chemical and Physical Agents. NATO Science Series. Amsterdam: IOS Press, 2000; 313:134-50.
17. Chandley AC. Maternal aging as the important factor in human aneuploidy. In: Dellaco VL, Voytek PE, Hollaender A, eds. Aneuploidy: Etiology and Mechanisms. New York : Plenum, 1985: 409-15.
18. Christman JK, Sheikhnejad G, Dizik M, Abileah S, Wainfan E. Reversibility of changes in nucleic acid methylation and gene expression induced in rat liver by severe dietary methyl deficiency. Carcinogenesis 1993; 14:551-7.
19. James SJ, Pogribna M, Pogribny IP, et al. Abnormal folate metabolism and mutation in the methylenetetrahydrofolate reductase gene may be maternal risk factors for Down syndrome. Am J Clin Nutr 1999; 70:495-501. Comment in: Am J Clin Nutr 1999; 70: 429-30.
20. Hobbs CA, Sherman SL, Yi P, et al. Polymorphisms in genes involved in folate metabolism as maternal risk factors for Down syndrome. Am J Hum Genet 2000; 67:623-30.
21. Kumar RK, Delatycki M. Down syndrome. In: Gupte S, ed. Recent advances in Paediatrics Neurology. New Delhi: Jaypee Brothers, 2001: 1-13.
22. Jones KL. Down syndrome (Trisomy 21 syndrome). In: Smith's recognizable patterns of human malformations. 5th ed. Philadelphia: WB Saunders Co, 1997: 8-13.
23. Fryns JP. Chromosome 21, trisomy 21. In: Buyse ML, ed. Birth defects encyclopaedia. USA: Center for Birth Defects Information Services, Inc. and Blackwell Scientific Publications, 1990:391-3.