Cytogenetic analysis of chromosomal abnormalities in Sri Lankan children

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Original article

Background: **Cytogenetic analysis is a valuable investigation in the diagnostic work up of children with suspected chromosomal disorders. The objective of this study was to describe the prevalence of various types of chromosomal abnormalities in Sri Lankan children undergoing cytogenetic analysis.**

Methods: **Cytogenetic reports of 1554 consecutive children with suspected chromosomal disorders who underwent karyotyping in two genetic centers in Sri Lanka from January 2006 to December 2011 were reviewed retrospectively.**

Results: **A total of 1548 children were successfully karyotyped. Abnormal karyotypes were found in 783 (50.6%) children. Numerical and structural abnormalities accounted for 90.8% and 9.2%, respectively. Down syndrome was the commonest aneuploidy identified. Other various autosomal and sex chromosomal aneuploidies as well as micro-deletion syndromes were also detected.**

Conclusions: **The prevalence of chromosomal abnormalities in Sri Lankan children undergoing cytogenetic analysis for suspected chromosomal disorders was relatively higher than that in Caucasian and other Asian populations.**

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Introduction

Tjio and Levan[1] found in 1956 that the normal chromosome complement in each human cell consisted of 46 chromosomes and not 48 as previously believed. Since then, various types of chromosomal abnormalities have been discovered as the underlying cause of numerous clinical syndromes and disease states in man. Chromosomal disorders may arise from either numerical and/or structural changes in the autosomes or sex chromosomes.[2] So far, approximately 1000 chromosome syndromes have been reported.[3] Cytogenetic analysis is an essential component in the diagnosis and evaluation of children with various congenital abnormalities, dysmorphic features, developmental delay and/or intellectual disability.[4]

Chromosomal aberrations are known to affect at least 75% of all conceptions and most of these are spontaneously aborted usually within the first trimester of gestation or end up as stillbirths thereafter.[5,6] It is estimated that chromosome abnormalities are present in up to 50% of first trimester abortions. The frequency of major chromosomal abnormalities is estimated to be between 1 in 150 to 1 in 200 live births.[2,7] According to Worton et al,[8] surveys conducted among healthy adult populations have found lower frequencies of chromosomal abnormalities (probably as a result of the high mortality among affected neonates and infants who fail to survive into adulthood). Studies found a wide range of chromosomal aberrations in children with suspected chromosomal disorders who were referred for cytogenetic analysis.[3,5,9,10] Although chromosomal disorders are recognized as one of the major causes of childhood morbidity and mortality in industrialized countries, these disorders have not received much attention in developing countries because of the prevailing burden of communicable and nutritional diseases.[5] As a result, the development of cytogenetic diagnostic facilities and provision of genetic counseling services in the public health sector of most developing countries has been relatively inadequate.

Although numerous studies have been conducted in Caucasian and other Asian populations, there is a paucity

of published data on the frequency of chromosomal abnormalities in Sri Lankan children referred for cytogenetic analysis. Jayasekara[11] studied the spectrum of chromosome anomalies and found a high proportion of trisomy 21 because of non-disjunction. He determined the prevalence of various types of chromosomal abnormalities in Sri Lankan children with suspected chromosomal disorders who were referred from various parts of the country for cytogenetic evaluation.

Methods

The study was carried out retrospectively at the Human Genetics Unit, Faculty of Medicine, University of Colombo and Asiri Center for Genomic and Regenerative Medicine, Asiri Surgical Hospital, Colombo, Sri Lanka. These are the only centers providing cytogenetic diagnostic services in Sri Lanka. Included in this study were children aged 12 years and below with suspected chromosomal disorders such as Down syndrome (DS), Turner syndrome (TS), Edward syndrome (ES), Patau syndrome (PS), disorders of sex development (DSD), and various other congenital abnormalities, dysmorphic features, intellectual disability and/or developmental delay, who were referred from all parts of Sri Lanka to the two centers for cytogenetic analysis between January 2006 and December 2011. Written informed consent for cytogenetic testing was obtained from the parents of all the children prior to testing. In order to determine whether the origin of some of the chromosomal abnormalities was *de novo* or familial, cytogenetic analysis of the parents was also performed after obtaining their consent.

Five milliliter sample of peripheral blood was obtained from each patient and chromosomal analysis was performed on routinely cultured lymphocytes after GTG-banding. Chromosomal analysis was done according to the guidelines of the International System for Human Cytogenetic Nomenclature (ISCN, 2009). At least 20 well-spread and well-banded metaphases were examined in each patient by an experienced cytogeneticist for numerical as well as structural abnormalities. The resolution of GTG-banding used was generally around 450-500 and the number of metaphase spreads examined in the case of mosaicism was 40. Standard descriptive statistics was used to analyze the data. Patients who were identified as having chromosomal abnormalities received post-test genetic counseling.

Results

A total of 1554 children with suspected chromosomal disorders were referred for cytogentic analysis. The age of the children ranged from birth to 12 years with a mean age of 1.9±3.2 years, and 897 (57.7%) children were aged below 1 year. Of the 1554 children, 786 (50.6%) were females and 768 (49.4%) were males. Samples from 1548 (99.6%) of the 1554 children were successfully analyzed. Of these samples, 783 (50.6%) were found to have abnormal karyotypes consisting of numerical abnormalities in 711 (45.9%) and structural abnormalities in 72 (4.7%). DS was the most common reason for referral for cytogenetic evaluation. The distribution of chromosomal abnormalities according to the referral reasons for cytogenetic analysis is shown in Table 1.

A total of 765 (49.2%) children were suspected of DS according to their clinical features and 763 (99.7%) children were successfully analyzed but 665 (86.9%) were karyotypically confirmed as having DS, i.e., 390 (58.6%) male and 275 (41.4%) female children with DS. The age of these children ranged from 1 day old to 11 years with a mean age of 0.7±1.7 years, and 496 (74.6%) children were aged less than 1 year. Free trisomy 21 due to non-disjunction was the commonest type found in 560 (84.2%) children, followed by mosaicism in 72 (10.8%) and translocation in 33 (5.0%). Translocation between chromosomes 14 and 21 was the commonest variety seen in 18/33 (54.5%) children, followed by translocation between the two chromosomes 21 in 8/33 (24.2%). The remaining 7 (21.2%) children had translocation between chromosomes 21 and 9, 13, 15 and 19. Translocations 21 and 9 and 21 and 19 both had features of DS. The maternal age of mothers of babies with translocation DS ranged from 20 to 43 years with a mean age of 29.3±6.3 years. The various types of chromosomal abnormalities in children referred with suspicion of DS are shown in Table 2.

There were 42 (2.7%) children with suspected ES and 18 (2.3%) were karyotypically confirmed as having ES. Seventeen (94.4%) children had free trisomy 18 due to non-disjunction, whereas 1 (5.6%) had a translocation between chromosomes 18 and 20. Fifteen (1.0%) children were suspected as having PS and 4 (0.5%) were karyotypically confirmed as having PS. Free trisomy 13 was found in 3 (75%) children,

Table 1. Distribution of chromosomal abnormalities according to the referral reasons for cytogenetic analysis

Reasons for referral	No. children referred *n* (%)	No. children with abnormal karyotypes *n* (%)
Down syndrome	765 (49.2)	665 (84.9)
Edward syndrome	42 (2.7)	18 (2.3)
Patau syndrome	15 (1.0)	4 (0.5)
Turner syndrome	149 (9.6)	50 (6.4)
Disorders of sexual development	230 (14.8)	12 (1.5)
Congenital anomalies, dysmorphic features, developmental delay, intellectual disability	353 (22.7)	34 (4.3)
Total	1554 (100)	783 (100)

Table 2. Chromosomal abnormalities in children referred with suspected Down syndrome

Karyotypes	No. children (*n*=665)	%
47,XX,+21	560	84.2
47,XY,+21		
46,XX/47,XX,+21	71	10.7
46,XY/47,XY,+21		
46,XX,rob(14;21)(q10;q10),+21 or 46,XY,rob(14;21)(q10;q10),+21	18	2.7
46,XX,rob(21;21)(q10;q10),+21 or 46,XY,rob(21;21)(q10;q10),+21	7	1.0
46,XX/46,XX,rob(21;21)(q10;q10),+21	1	0.2
46,XY,rob(13;21)(q10;q10),+21	3	0.4
46,XX,rob(15;21)(q10;q10),+21	2	0.3
46,XY,t(19;21)(q10;q10),+21	1	0.2
46,XX,t(9;21)(q10;q10),+21	1	0.2
47,XY,+21/48,XY,+21,+mar	1	0.2
Total	665	100.0

but the remaining children had translocation between chromosomes 13 and 9.

A total of 149 (9.6%) children were referred with the suspicion of TS and 50 (6.4%) were found to have abnormal karyotypes. The mean age of children referred for cytogenetic analysis was 4.5±4.5 years. TS variants were found to be more common than the classical 45,X monosomy [27 (54.0%) versus 23 (46.0%)]. The TS variants included: 45,X/46,XX [34.0%], 45,X/46,X,i(X)(q10) [8.0%], 45,X/47,XXX [2.0%], 45,X/46,XX/47,XXX [2.0%], 45,X/46,XX/47,XXY [2.0%], 46,X,i(X)(q10) [2.0%], 46,X,idic(X)(q22) [2.0%] and 45,X/46,X,der(X)t(X;Y) [2.0%].

A total of 230 (14.8%) children with DSD were referred for karyotype assessment and 229 (99.6%) were successfully karyotyped. The mean age of children referred for karyotype assessment was 2.6±4.4 years. Male karyotype (46,XY) was found in 133 (58.1%) and female karyotype (46,XX) in 84 (36.7%) children. XY females accounted for 7 out of 133 (5.3%) children with a male karyotype. Various chromosomal abnormalities were observed in children referred for investigation of DSD such as 46,XX/46,XY [1.7%], 45,X/46,XY [1.3%], 46,i(Xq)Y [0.4%], 46,XX,inv(9)(p11q11) [0.4%], 46,XY,t(6;22) [0.4%], 46,XY,t(9;11)(p24;q22) [0.4%] and 46,X,del(Y)(q11) [0.4%].

Among the 353 (22.7%) children with various congenital abnormalities, dysmorphic features, developmental delay and/or intellectual disability, 34 (4.3%) were found to have abnormal karyotypes with numerical abnormalities in 7 (20.6%) and structural abnormalities in the remaining 27 (79.4%). The chromosomal abnormalities in children referred for investigation of various congenital abnormalities, dysmorphic features, developmental delay and/or intellectual disability are shown in Table 3.

Table 3. Chromosomal abnormalities in children referred for investigation of congenital anomalies, dysmorphic features, developmental delay and/or intellectual disability

Karyotypes	No. children (*n*=34)	%
Numerical abnormalities		
49,XXXXY	2	5.9
47,XYY	1	2.9
47,XXY	1	2.9
47,XXX	1	2.9
45,X/46,XY	1	2.9
47,XXY/46,XY	1	2.9
Structural abnormalities		
46,XY,rob(13;14) (q10;q10) or 46,XX,rob(13;14)(q10;q10)	2	5.9
45,XX,rob(13;14) (q12.1;q11.2)	2	5.9
46,XX,del(5)(p15.2pter)	3	8.8
46,XX,der(5)add(8)(q13)del(5)(p13) pat	1	2.9
46,XX,der(22)t(5;22)(p12;q11.2)(06)/46,XX(10)	1	2.9
46,XY,der(18)t(8;18)mat	1	2.9
46,XX,del(18)(p11.2pter)	1	2.9
46,XX,del(18)(q21.31qter)	1	2.9
46,XY(6)/47,XY,+mar(18)	1	2.9
46,XX/46,XX,del(14)(q11.2q13)	1	2.9
46,XX,inv(22)(p11.2q12.3)pat	1	2.9
45,XX,t(19;22)/46,XX/47,XX,+21	1	2.9
46,XX,+inv dup(12)(p11.2pter)	1	2.9
46,XX,der(15)t(3;15)(p24;q26)mat	1	2.9
46,XY,del(9)(q13q21)	1	2.9
46,XY,del(9)(p22pter)(37)/47,XY, del(9)(p22pter),+mar(63)	1	2.9
46,XY,del(11)(q23.2qter)	1	2.9
46,X,del(Y)(q11)	1	2.9
46,XX,?del (12)(q23)	1	2.9
46,XX,dup(17)(q24q25)	1	2.9
46,XY,del (15)(q11.2q12)	1	2.9
46,XX,del(4)(p15.3pter)	1	2.9
47,XX,+mar	1	2.9
Total	34	100

Three patients with marker chromosomes were identified, but the origin of the additional chromosomal fragments could not be determined by conventional GTG-banding. The origin of these chromosomal fragments can only be verified by the use of molecular cytogenetic methods such as fluorescence *in situ* hybridization (FISH) and micro-array which at the moment have not yet been established at the 2 cytogenetic diagnostic centers in this country.

Discussion

In this study, the prevalence of chromosomal abnormalities was found to be 50.6% in children. The prevalence of chromosomal abnormalities in Sri Lankan children has been compared with Caucasian and other

Asian populations.[3,5,9,10,12] The high prevalence in the present study is probably due to the fact that the study was carried out exclusively among children who were referred for cytogenetic analysis of suspected chromosomal disorders. Even though prenatal diagnostic techniques such as amniocentesis, cordocentesis, ultrasonography and maternal serum screening are available in this country, the continuing legal prohibition on termination of pregnancies for fetal indications implies that the prevalence of children born with chromosomal disorders will continue to be high. This may be one of the main reasons for the high prevalence of chromosomal abnormalities in children in this study. Other reasons for the difference in prevalence reported in these studies may be due to the varied inclusion criteria of patients, the cytogenetic methods used and the discordance of classification criteria.[5] Both adults and children were included in other studies[3,5,9,12-14] and only few were conducted exclusively among children suspected of chromosomal disorders.[15,16] The majority of chromosomal abnormalities observed in this study were numerical abnormalities (45.9%). A similar preponderance of numerical abnormalities was found in a study of 4216 patients.[3]

The results of this study indicate the importance of cytogenetic analysis of children suspected of chromosomal disorders. Chromosomal analysis is essential for establishing a definitive diagnosis, deciding clinical management, and estimating the risk of recurrence of chromosomal disorders in future pregnancies, and providing appropriate genetic counseling. It is also helpful in identifying structural chromosomal aberrations which are likely to be familial. In such cases, chromosomal analysis of the parents is warranted because they are possibly carriers of a balanced structural chromosomal aberration.

Autosomal abnormalities

In this study, DS was the commonest autosomal aneuploidy and the most frequent reason for cytogenetic analysis, and the majority of such patients were karyotypically confirmed as having DS. The findings in this study are in agreement with a previous study conducted by Jayasekara in 1988 on the spectrum of chromosome anomalies seen at the Human Genetics Unit, Faculty of Medicine, Colombo.[11] He reported a high proportion (76.3%) of Down syndrome cases among the 76 patients with chromosome anomalies. Similar findings were obtained in recent studies conducted in Southeast Turkey by Balkan et al on 4216 patients and in South Korea by Kim et al on 4117 patients suspected of chromosomal abnormalities.[3,9] Among the DS children, there was a male predominance (58.6% males versus 41.4% females). In a meta-analysis of data from 55 independent studies, Kovaleva[17] reported a similar male preponderance among DS patients. Possible genetic mechanisms of male predominance in trisomy 21 include the joint segregation of chromosome 21 and Y chromosome in spermatogenesis and the chromosome 21 non-disjunction during second meiotic division in oogenesis caused by Y chromosome-bearing spermatozoa.[17] A majority (74.6%) of children with karyotypically confirmed DS were aged less than 1 year. Balkan et al[3] also reported that nearly 70% of the DS cases in their study were aged less than one year. This indicates early referral by the investigating clinicians for chromosomal analysis which may have resulted from a high index of clinical suspicion as well as from the increased awareness among clinicians about cytogenetic analysis. In agreement with several previous studies,[3,9,11,14,16,18] chromosomal non-disjunction was the main cause of DS with 84.2% of the children having free trisomy 21. DS due to mosaicism (10.8%) was found to be higher than due to Robertsonian translocations between chromosome 21 and the acrocentric chromosomes (5.0%). Similar findings were reported in a recent study on 1001 DS cases conducted by Jyothy et al in Andhra Pradesh, India.[19] In the study, the frequency of pure trisomy, mosaicism and translocation was 87.9%, 7.7% and 4.4% respectively. However, studies conducted by Kim et al[9] in South Korea, Ahmed et al[16] in Pakistan and Verma et al[18] in India all reported higher frequencies of translocation DS than the mosaic form. It is interesting to note that the cytogenetic pattern of DS is variable among different studies.[9,14,16,18,19] It is difficult to identify the reasons for these discrepancies but could be due to the variations in the selected study populations. Identification of translocation DS in the fetus or newborn is an indication for karyotypic analysis of both parents as either of them may be carriers of a balanced translocation involving chromosome 21. Translocation carriers have a high risk of aneuploid offspring with every pregnancy, the recurrence risk depends on the sex of the carrier parents and the chromosomes that are fused.[20] If one of the parents is the carrier of a balanced translocation involving the two chromosome 21s, the recurrence risk for DS is 100%. Nance and Engel[2] suggested that translocation DS should be suspected especially when the proband is the offspring of a young mother under 25 years of age and also in instances when there is a history of DS in the family. However, in this study, the maternal age of mothers of babies with translocation DS was in the range of 20 to 43 years with a mean age of 29 years. In addition to indicating the recurrence risks of the syndrome, Santos et al[14] noted that cytogenetic analysis can be helpful in the clinical follow-up of some disorders associated with DS such as acute leukemia

and Alzheimer's disease leading to early diagnosis and treatment of these conditions. Thus, all children with a clinical diagnosis of DS should be referred for cytogenetic confirmation and genetic counseling.[20]

Patau syndrome may occur as a freestanding trisomy 13 or more rarely, as a Robertsonian translocation with an extra copy of chromosome 13 attached to one of the acrocentric chromosomes e.g. 13-15, 21, 22 or as a structural chromosome abnormality wherein only a part of chromosome 13 is duplicated. In this study, non-disjunction was found to be the predominant cause of both Patau syndrome (Trisomy13) and Edward syndrome (Trisomy18) and no cases of somatic mosaicism were identified.

Sex chromosomal abnormalities

Only 5.3% of children had a discrepancy between the genetic sex and the phenotypic sex. They had normal female phenotype with 46,XY chromosome complement suggestive of sex reversal conditions. This percent was relatively lower than those found in several Indian studies.[12,21] Rajasekhar et al[12] reported a higher proportion (14.9%) of XY females among 1400 referral cases in India, whereas another study[21] on 30 Indian patients with disorders of sex development in Coimbatore city identified 10 (33.3%) XY females. The study population in both studies included children and adults who may have contributed to the higher frequencies reported.

TS was the commonest sex chromosomal aneuploidy accounting for 9.6% of cases and 6.4% of them were karyotypically confirmed as having TS. TS usually results from total or partial absence of one of the two X chromosomes normally present in females. It may also result from a structurally abnormal X chromosome in which deletion or duplication of genetic material has occurred. TS is commonly diagnosed at puberty because of failure of sexual maturation resulting from ovarian dysgenesis.[12] However, an increasing number of patients are now being recognized during infancy and childhood because of clinicians' increased awareness of other stigmata such as peripheral lymphedema, growth failure, short stature, webbed neck, shield chest, low posterior hairline, pigmented nevi, hypoplastic nails, short fourth metacarpals and coarctation of the aorta.[2] In this study, TS variants (54.0%) were found to be commoner than the classic 45,X karyotype (46.0%). Various mosaic forms in association with a 45,X cell line were the commonest TS variants seen in 50% of cases. Similar findings were reported by Duarte et al[5] in a study on 916 patients from all age groups where TS mosaicism (53.6%) was commoner than monosomy TS (28.6%). Kim et al[9] in South Korea and Rajasekhar et al[12] in India also reported that the proportion of TS mosaics was higher than that of classic TS. However, an Indian study[22] found that in 45 cases of TS, the most commonly observed karyotype was 45,X (44.4%), followed by 45,X/46,XX mosaicism (24.4%). These variations could possibly be explained by the differences in the referral reasons of the study populations.

Chromosomal abnormalities in children with congenital anomalies, dysmorphism, developmental delay and/or intellectual disability

Among the 353 children with various congenital abnormalities, developmental delay, dysmorphic features and/or intellectual disability, 34 (4.3%) had a variety of chromosomal aberrations detected by conventional GTG-banded cytogenetic analysis, such as unbalanced translocations, deletions, duplications, inversions and marker chromosomes. A higher prevalence was found in 98 children with congenital malformations and intellectual disability,[14] of whom 26% had abnormal karyotypes. A recent study[3] found chromosomal abnormalities in 13.6% of 568 children with intellectual disability, dysmorphic features, congenital anomalies and developmental delay. In this study, 4 children were diagnosed with a terminal deletion of 5p characteristic of Cri-du-chat syndrome [del(5)(p15.2pter)], 1 child with Wolf-Hirschhorn syndrome [del(4)(p15.3pter)], 1 with Jacobsen syndrome [del(11)(q23.2qter)] and another child suspected with Angelman syndrome [del(15)(q11.2q12)] based on their clinical features. Yashwanth et al[15] found that evaluation of chromosomal abnormalities is important in understanding the underlying etiology of congenital malformations and intellectual disability. However, clinical diagnosis with molecular cytogenetic techniques (fluorescence in situ hybridization (FISH) and micro-array) in such patients could be improved.[14]

In conclusion, a variety of chromosomal abnormalities were identified in Sri Lankan children undergoing cytogenetic analysis. This demonstrates the importance of cytogenetic evaluation in children with various congenital abnormalities, dysmorphic features, developmental delay and/or intellectual disability. The types of chromosomal abnormalities identified in this study were similar to those found in other studies.

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