Original Article

DOI: 10.7860/NJLM/2025/74501.2896

Pathology Section

A Cross-sectional Study on Molecular Cartography: The Mapping of Down Syndrome with Cytogenetic Tools

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ABSTRACT

Introduction: Down Syndrome (DS), or trisomy 21, is the most common genetic cause of intellectual disability among children, with an incidence of 1 in 700 births. This extra copy of chromosome 21 leads to characteristic clinical features known as Down facies, which include microcephaly, hypertelorism, a flat nasal bridge, macroglossia, a fissured tongue, microophthalmia, large ears, and various physical abnormalities like short stature, a short neck, a sandal gap, and a single palmar crease. Additionally, individuals with DS often experience intellectual disabilities, including delayed speech development and slow learning. Although the characteristic phenotypes are available for diagnosing DS, genetic testing is necessary for confirmation.

Aim: To confirm the diagnosis of DS through karyotyping, a cytogenetic analysis.

Materials and Methods: This cross-sectional study was conducted in the Department of Pathology (Molecular Pathology Laboratory) at the Institute of Child Health, Madras Medical College, Chennai, Tamil Nadu, India over a period of eight months from September 2023 to April 2024. The study included 50 patients, all children aged between 10 days and six years, who exhibited clinical features of DS. A peripheral blood sample of 3 mL was collected in a heparinised vacutainer. Karyotyping was performed, and the samples were stained with Giemsa banding using trypsin digestion. Metaphase spreads were examined under a microscope (Olympus) with the assistance of Applied Spectral Imaging (ASI) software, and karyotype descriptions were made according to the International Standard Committee on Human Cytogenetics Nomenclature guidelines. Descriptive analyses were conducted using Statistical Package for Social Sciences (SPSS) software version 29.0.2. Parameters like age, gender, risk factors (including elderly mothers and children born to consanguineous parents), and common associations (such as cardiovascular abnormalities) were analysed.

Results: Among the 50 children with clinical features of DS, a genotypic correlation was found in 48 children (96%). Among these, pure trisomy 21 was identified in 34 children (70%), Robertsonian translocation in 9 (20%) children, and mosaics in 5 (10%) children. The cardiovascular system was the most commonly affected system, with 38 (80%) children showing abnormalities, and 28 (60%) children were born to elderly primiparous mothers, making this the most frequently observed risk factor.

Conclusion: Despite advancements in non invasive prenatal screening techniques, karyotyping remains a definitive and widely used method for diagnosing DS. Continued research in this field aims to improve early detection and expand the understanding of the genetic mechanisms underlying the disorder.

Keywords: Karyotyping, Trisomy 21, Translocation

INTRODUCTION

Dr. John Langdon Down was the first person to describe the syndrome, which is now known as DS or trisomy 21 [1]. This genetic disorder is characterised by an extra copy of chromosome 21 and occurs in approximately 1 in 700 babies [2-6]. This genetic anomaly leads to a range of developmental and intellectual disabilities, which include low-set ears, epicanthal folds, a sandal gap, a depressed nasal bridge [7-9], simian crease, a short neck, a flat nose, almond-shaped eyes, upward slanting, palmar crease, poor muscle tone, short stature, and congenital heart disease, including ventricular septal defect, patent ductus arteriosus, and to some extent, atrial septal defect. Neurological abnormalities may include intellectual disability [10], delayed language and speech development, slow learning, seizure disorders, dystonia, and psychiatric disorders that may manifest at a later age. Haematological disorders associated with DS include acute myeloid leukaemia and transient abnormal myelopoiesis.

Cytogenetic analysis plays a pivotal role in understanding the genetic basis of DS. Karyotyping, the most common cytogenetic technique, provides a visual representation of chromosomes, allowing for the detection of trisomy 21 [11] and other chromosomal disorders like Klinefelter syndrome, Patau syndrome (trisomy 13), Edward syndrome (trisomy 18), Turner syndrome, and structural abnormalities including deletions, duplications, and translocations.

The present study aimed to confirm the diagnosis of DS through karyotyping in patients exhibiting the clinical features of the condition and to categorise the types of chromosomal abnormalities. Through cytogenetic analysis, researchers and clinicians can gain insights into the genetic diversity and variability associated with DS, facilitating accurate diagnosis, genetic counselling, and management strategies for affected individuals. The present article explores the methodologies, findings, and clinical implications of cytogenetic studies in DS, highlighting their significance in the broader context of genetic research and clinical settings.

MATERIALS AND METHODS

The present cross-sectional study was conducted in the Department of Pathology (Molecular Pathology Laboratory), Institute of Child Health, Madras Medical College, Chennai, Tamil Nadu, India over a period of eight months from September 2023 to April 2024 after obtaining approval from the Institutional Ethical Committee (IEC NO-56092024).

Inclusion criteria: In present study, a total of 50 children with clinical features of DS- such as Down facies, short stature, palmar crease, sandal gap, poor muscle tone, delayed language development, poor learning ability, intellectual disability, seizure disorder, and

congenital heart diseases like patent ductus arteriosus, ventricular septal defect, and atrial septal defect-aged between 10 days and six years were included.

Exclusion criteria: Phenotypically and genotypically normal children were excluded from the present study. Participants were selected based on the above mentioned inclusion and exclusion criteria.

Study Procedure

All socio-demographic, anthropometric, and investigative data were obtained from the patients' medical records. Clinical history, including family history of DS, paternal and maternal age, and consanguinity, was obtained from the parents.

Sample collection: A peripheral blood sample of 3 mL was collected from patients in a heparinised vacutainer.

Culture and harvesting: Authors added 28 drops of sodium heparinised blood to 5 mL of complete medium {which contains RPMI 1640 medium, Foetal Bovine Serum (FBS) and Phytohaemagglutinin (PHA)} in a 15 mL centrifuge tube. Under aseptic conditions, the mixture was incubated for 67 hours at 37 degrees Celsius, releasing CO_2 every 24 hours. Then, 50 µL of colchicine (0.01%) was added, and the sample was incubated for an additional 20 minutes. After this, the sample was spun down at 1500 rpm for six minutes. The supernatant was discarded, and immediately 6 mL of KCl was added, followed by incubation for 20 minutes. Next, 2 mL of freshly prepared cold fixative (methanol and glacial acetic acid) was added. The sample was then spun down at 1500 rpm for six minutes, and the supernatant was discarded. An additional 6 mL of cold fixative was added to the pellet and left at room temperature for 3-4 hours. The sample was spun down again at 1500 rpm for six minutes, and the supernatant was discarded. Finally, 6 mL of freshly prepared fixative was added, and the sample was refrigerated overnight.

Slide preparation: The slides were prepared using the drop-down method on ice-cold slides and incubated. The slides were aged for three days.

Banding (Trypsin digestion): Depending on the age of the slides, the slides were dipped in trypsin solution for 3-5 seconds. After that, the slides were dipped in cold normal saline to stop the trypsin activity and washed under tap water. They were then placed in Giemsa solution for 5-7 minutes and washed again in tap water. After drying, the slides were mounted with a cover slip using DPX solution.

Metaphase analysis: Metaphase was examined under a microscope (Olympus) with the assistance of ASI software. For present study, we examined 25 metaphases, and one cell line was photographed and karyotyped. In cases of mosaicism, 50 metaphases and 2-3 cell lines were scored. The karyotype description was conducted according to the international nomenclature guidelines established by the International Standard Committee on Human Cytogenetics Nomenclature (ISCN 2016) [12].

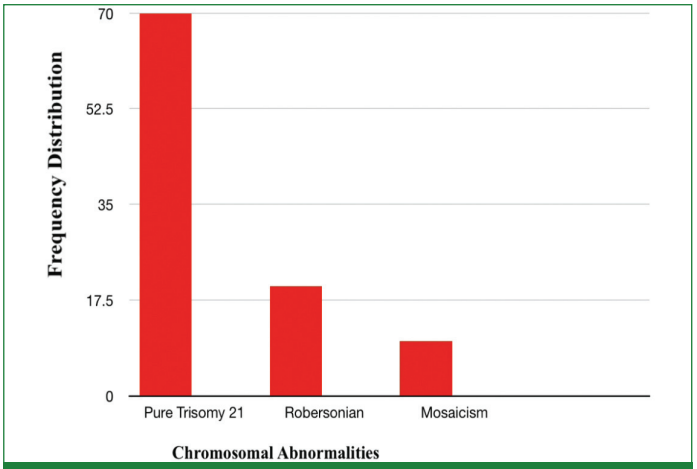
STATISTICAL ANALYSIS

The analysis was performed using SPSS software, version 29.0.2. Descriptive statistics were applied, and results were presented as percentages to summarise the distribution of variables.

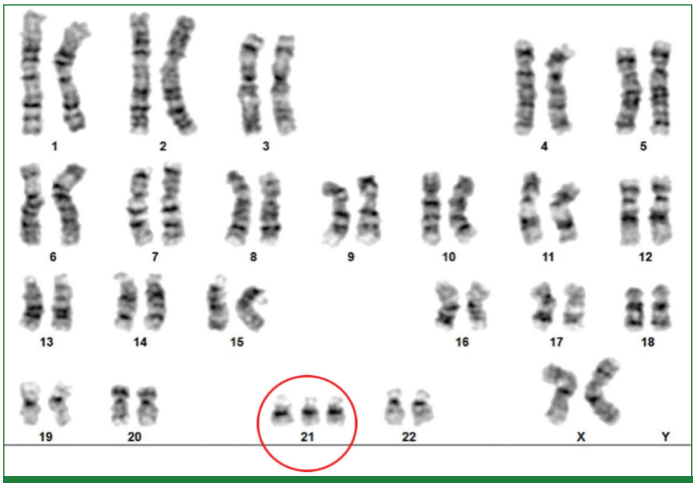
RESULTS

In present study, the diagnosis of DS was confirmed by karyotyping. Out of 50 children, 48 (96%) were found to be genotypically positive, while 2 (4%) were negative for DS has been depicted in [Table/Fig-1]. Among those, 34 children (70%) had pure trisomy 21 [Table/Fig-2], 9 (20%) children were mosaics [Table/Fig-3], and 5 (10%) children had Robertsonian translocation [Table/Fig-4].

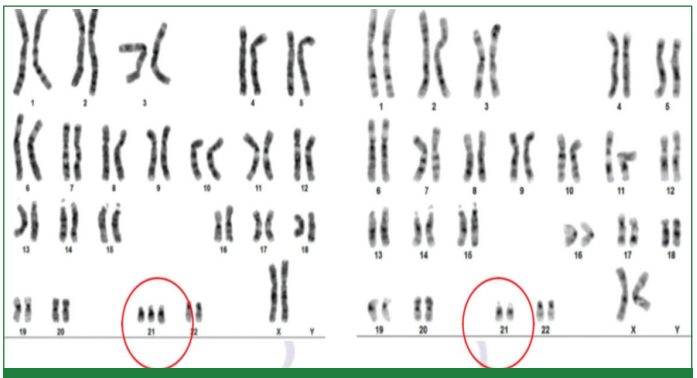
In present study, girls were more frequently affected than boys, with 31 (65%) girls compared to boys. Children aged less than one year were most frequently affected, with 29 (60%) children in this age group, compared to 19 (40%) children in the age group of more than one year. Furthermore, children born to older mothers were more commonly affected, with 36 (75%) children compared to those born to younger mothers.



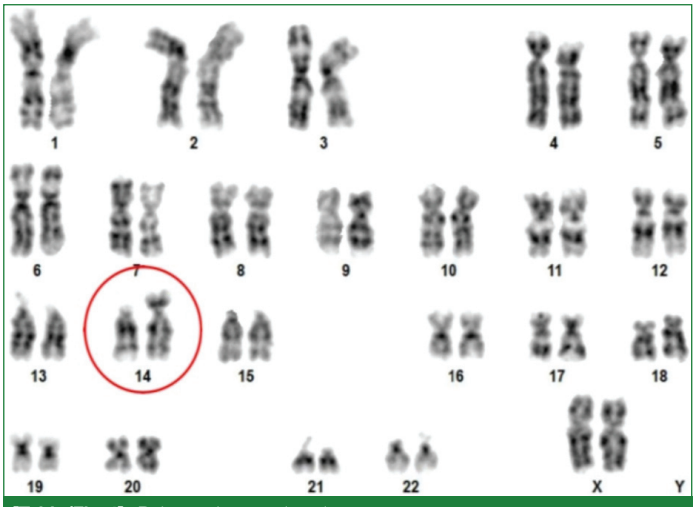
[Table/Fig-1]: Chromosomal abnormalities in Down Syndrome.



[Table/Fig-2]: Pure trisomy 21.



[Table/Fig-3]: Mosaicism.



[Table/Fig-4]: Robersonian translocation.

The present study, which included 48 children with DS, revealed that 38 children (80%) primarily presented with cardiovascular abnormalities, with ventricular septal defect being the most commonly observed congenital heart defect. The remaining 10 children (20%) displayed central nervous system anomalies. Moreover, the occurrence of congenital abnormalities was higher among the 36 children (75%) born to parents in consanguineous marriages compared to those born to non-consanguineous parents.

DISCUSSION

Down syndrome is the most common genetic cause of intellectual disability among children, and its incidence is rapidly increasing. Therefore, the confirmation of a genotypic diagnosis through cytogenetic analysis is necessary to prepare for a multidisciplinary treatment protocol [13]. The mapping of phenotypes to specific regions of chromosome 21 allows for the identification of the genes that contribute to the phenotypic features of DS. Hence, karyotype analysis remains a cornerstone in the diagnosis.

A prevalence distribution of chromosomal abnormalities in Chinese DS patients in Nanning [14] is similar to present study, with pure trisomy 21 [15-17] being the most common, observed in 38 children (70%), followed by Robertsonian translocation in 9 (20%) children and mosaicism in 5 (10%) children, which is the least common. The present finding is also in concordance with other studies, such as that by Nigam N et al., which included 30 patients; among them, 28 (93.75%) children had pure trisomy 21, while the remaining 2 (6.25%) children had Robertsonian translocation [18].

In a study conducted by Verma PK et al., at AIIMS, Rishikesh, among 40 children with DS, 39 of them (90.7%) had pure trisomy while 1 (2.5%) child had Robertsonian translocation, with no cases of mosaicism reported [19].

Similarly, in a study by Sharath K et al., at Sri Siddhartha Medical College, Karnataka, involving 75 children with DS confirmed via karyotyping, 59 children (78.7%) were diagnosed with pure Trisomy, 11 with translocations (8%), and 2 children (2.6%) with mosaicism [20].

In a larger cohort study by Jaiswal SK et al., at Banaras Hindu University, out of 436 children with DS, 416 (95.3%) were found to have pure trisomy, 13 children (3%) had Robertsonian translocation, and 7 (1.6%) children were diagnosed with mosaicism [Table/Fig-5] [21].

Author	Total no	Pure trisomy 21	Translocation	Mosaics	Place of study
Nigam N et al., 2019 [18]	30	29 (93.75%)	1 (6.25%)	-	Lucknow
Verma PK et al., 2022 [19]	40	39 (90.70%)	1 (2.50%)	-	Rishikesh
Sharath K et al., 2018 [20]	75	59 (78.7%)	11 (14.7%)	2 (2.6%)	Karnataka
Jaiswal SK et al., 2021 [21]	436	416 (95.3%	13 (3%)	7 (1.6%)	Uttar Pradesh
Current study, 2024	50	70%	20%	10%	Chennai

[Table/Fig-5]: Karyotype frequencies among Down syndrome cases in other studies [18-21].

In present study, out of 48 children with DS, the most common association was with the cardiovascular system, seen in 38 children (80%), compared to central nervous system involvement in 10 children (20%). A retrospective analytical study conducted by Kava MP et al., included a sample of 524 patients, where cardiovascular system abnormalities were found to be most common, aligning closely with the current study, which reported 80% of cardiovascular abnormalities in 419 children and 105 children (20%) with central nervous system abnormalities [22].

According to a study conducted by Verma Ic et al., in Libya and Berisha SZ et al., were showed the risk factors of antenatal mothers like increased maternal age, represented 82% of the cases. This is similar to the results of the current study, where 28 children (60%) were born to high-risk mothers, including those with advanced maternal age and gestational diabetes managed with insulin [23,24].

Limitation(s)

The current study is limited by a small sample size, and cross-sectional studies capture data at a single point in time, which limits the ability to infer causality or observe changes over time. Moreover, findings from a specific population may not be applicable to other populations, and the results may have limited relevance outside the studied group, which restricts their broader application. Additionally, the accuracy and resolution of the karyotyping techniques used may limit the detection of subtle chromosomal abnormalities. This could lead to underreporting or mischaracterisation of certain genetic variations.

CONCLUSION(S)

The present study underscores the vital role of chromosomal analysis in diagnosing DS and identifying variations like trisomy, translocation, and mosaicism, which impact recurrence risk and clinical outcomes. Mosaicism presents the lowest recurrence risk, while translocation has a higher risk. Therefore, genetic counselling and prenatal screening are key to preventing future cases. Early karyotyping can significantly improve health management for affected children. Future research, using advanced genomic tools, should explore larger and more diverse populations to deepen present understanding and enhance care for individuals with DS.

Acknowledgement

Authors would like to express their gratitude to cytogenetics Cytogenetics Laboratory, Department of Pathology, Institute of Child Health and Hospital for Children, Chennai, Tamil Nadu, India for their contributions.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Jul 26, 2024
- Manual Googling: Sep 24, 2024
- iThenticate Software: Oct 21, 2024 (9%)

ETYMOLOGY: Author Origin

EMENDATIONS: 8

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Jul 25, 2024**
Date of Peer Review: **Aug 14, 2024**
Date of Acceptance: **Oct 22, 2024**
Date of Publishing: **Jan 01, 2025**