World Journal of Biology Pharmacy and Health Sciences
eISSN: 2582-5542
Cross Ref DOI: 10.30574/wjbphs
Journal homepage: https://wjbphs.com/

(RESEARCH ARTICLE)

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Molecular cytogenetic analysis using FISH and HD-oligo micro array identified an unusual novel Down syndrome case

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World Journal of Biology Pharmacy and Health Sciences, 2023, 14(02), 320–328

Publication history: Received on 09 April 2023; revised on 26 May 2023; accepted on 28 May 2023

Article DOI: https://doi.org/10.30574/wjbphs.2023.14.2.0227

Abstract

Background: Down syndrome, caused due to trisomy of chromosome 21.

Methods: 131 suspected cases of DS from university hospital after obtaining written informed consent clinical history and photographs were also recorded. Diagnosis carried out by tacking peripheral blood from the children, setting up whole blood culture as well as DNA extraction from leukocytes for molecular analysis. G-banding by Saline-Trypsin-Giemsa (STG) method and Karyotyping.

Results: They were into four categories using automated Karyotyper. **Category 1**, out of 131 96(73.28%) cases of these had trisomy 21 while **Category 2,** showed 3(2.29%) cases with translocation or isochromosome involving chromosome 21. **Category 3,** was 0 mosaics cases. **Category 4,** was 0, will be karyotypically 46,/XY but show partial duplications case. An unusual Down syndrome is **Category 5,** had remaining 32(24.42%) cases, though referred for DS were karyotypically 46,XX or 46,XY. Of these only one cases were judiciously selected and tested for partial duplication of Down syndrome critical region (DSCR) with the help of FISH by using LSI 21 chromosome 21 specific probe but as such no duplication was observed. One of the above two case was further investigated through oligo-based cytogenetic array for micro-duplication or deletion of region on chromosome 21 other than the DSCR and it revealed no duplication or deletion of any part of chromosome 21 or whole genome.

Conclusion: This study suggested besides trisomy 21 and chromosomal rearrangements there might be genetic imbalances leading to perturbations of pathway/s could result unusual Down syndrome.

Keywords: Down syndrome; Mongoloid face; Trisomy 21; FISH; Oligo-based cytogenetic array; BAC probe

1. Introduction

Down Syndrome generally caused by presence of three copies of chromosome 21 (trisomy 21) is one of the most common chromosomal disorders with an incidence of 1 in 700 live births. Phenotypic manifestation due to trisomy 21 varies from very mild to severe form. Among trisomy 21 cases, 95% of the cases have free trisomy 21 and remaining 5% cases have Robertsonian translocation, isochromosome, mosaicism and partial duplication. In 95% of the trisomy 21 cases, the extra copy of chromosome 21 was reported to be maternal in origin (Antonarakis, 1993). Studies suggest that 80% of DS cases occur due to non-disjunction of chromosome 21 occurring at meiosis I stage of gamete formation when mothers are in their embryonic developmental stage. Advanced maternal age and altered recombination frequency had been considered as known risk factors for predisposition of mothers for non-disjunction of the chromosomes (Lamb et al. 1996; Morris et al.2002). Though, high incidence of the cases in younger mothers revealed that along with advanced maternal age, other factors are also involved in non-disjunction of the chromosomes. Exact

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mechanism responsible for non-disjunction of chromosome 21 in younger mothers (less than 35 years) has not been explored yet.

1.1. Clinical phenotypes of Down Syndrome

Down syndrome shows most characteristic facial patterns (rounded face, epicanthic folds, protruding tongue, drooping neck) right from the birth that facilitates their recognition. However, nearly 80 different features have been identified in DS, not all of them occurring in a case (Epstein et al. 1991, 2002). Some of the more common morphological features (**Figure 1**) include single simian crease, flattened nasal root, midfacial hypoplasia, wide gap between 1st and 2nd toe (sandal toe) etc. (Epstein et al. 1986). In addition to these, there are more severe pathological features. Mild to severe mental retardation is common to 100% DS patients, the IQ ranging from 20 to 80 and asthmatic symptoms occur in around 30% of them (Carr 1995). Most early deaths in DS occur due to congenital heart defects. There are other problems such as gastrointestinal defects caused because of constriction of duodenum or esophagus. Hypotonia is noticed in around 100% of the cases. Early onset of Alzheimer's disease, which is usually a late age short-term memory loss disorder, is common in DS (Tolmie 2002, Epstein et al (1991).

Figure 1 Phenotypic similarities between different chromosomal subtypes.

1.2. Molecular cytogenetics of Down syndrome:

The chromosomal constitution of a typical Down syndrome was first published by Lejeune et al. (1959), which showed 47 chromosomes with an additional group G chromosome. Chromosomal data show that more than 95% DS are caused by trisomy of chromosome 21 (Fryns 1987). However nearly 5% of DS become familial because of translocation of chromosome 21 to another chromosome. The origin of a metacentric chromosome 21 is rarely due to translocation between the two homologues, rather it arises as an isochromosome of 21q (Grasso et al. 1989, Shaffer et al. 1993).

Another category of DS is mosaicism for chromosome 21 trisomy. The proband in these cases has at least two cell lines, one with the normal chromosome complement and the other with the trisomy. Obviously unlike the trisomy where the zygote itself is aneuploid, in the mosaics a normally diploid zygote undergoes a nondisjunction during an early cell division stage, giving rise to more than one cell line. The frequency of different cell types depends upon the cell number at which the nondisjunction occurred, and on the differential selection of the cells (Antonarakis et al. 1993). However, there is no striking difference in the etiology of the DS arising out of trisomy or as mosaics.

In addition to these, there are also cases of DS with no chromosomal anomaly in number or structural rearrangement. Studies on these revealed small duplication within the chromosome itself 21q22(qter). Since trisomy of a small part of 21 could elicit the DS symptoms, it led to the idea that not whole but partial trisomy was enough to cause DS (Kubien, 1978). The region around 21q22 was suspected to the Down syndrome critical region (Nadal et al. 2001). However, despite the whole chromosome sequencing, no highly critical genes have been identified in this region to account for all the various phenotypes associated with DS (Delabar et al. 1993). Models for understanding the DS critical regions, mouse genome has been analysed (Kola et al. 1997) and the long arm of chromosome 16 in mouse shows a great deal of synteny with chromosome 21 (Sago et al. 1998, Richtsmeier et al. 2000). Trisomy of partial chromosome 16 in mouse does result in failure of a number of cognitive features comparable to those seen in DS (Shinohara et al. 2001. Molecular markers through the length of chromosome 21 have been developed in the last 25 years and molecular maps of ~5cM distance have been created to employ for gene mapping and other related studies (McInnis et al.1993). These markers have been of use in mapping individual disease genes but have not helped in revealing a DS sensitive region (Hassold at al. 2001). Similarly, annotation of chromosome 21, following the resolution of its sequence (Hattori et al. 2000) reveals a number of novel genes on various regions of the chromosome. However, any set of genes as a stronger candidate for

DS has not been identified (Gardiner et al. 2002, Reymond et al. 2002). The trisomy of chromosome 21 category. And even though partial trisomy has also shown DS phenotypes, a critical region has not been precisely mapped.

2. Material and methods

2.1. Subjects

A total of 131 suspected cases of DS were registered in present study. All the cases were referred from Sir Sunder Lal Hospital, Institute of Medical Sciences, Banaras Hindu University, Varanasi. The period of sample collection was from 20011 to 2015. Clinical photographs and clinical history of each case was recorded in checklist having major 36 DS clinical features. Medical history of parents including their ethnicity, age, previous incidence of any abortions and birth of any child with congenital anomalies were recorded. Control mothers were recruited from the Outpatient clinic of the Gynecology Department, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India. All patients belonged to Eastern Uttar Pradesh and the contiguous regions of Bihar, Jharkhand and Chhattisgarh. About 5 ml peripheral blood sample was drawn in heparinized syringe under complete aseptic condition from mother, father, and child after obtaining a written consent. This study was approved by the Institutional Ethical Committee of the Institute of Medical Sciences, Banaras Hindu University, Varanasi, India.

2.2. Whole blood culture

Whole blood culture was set in RPMI-1640 pH 7.2 (Sigma-Aldrich, Inc., St. Louis, MI, USA) culture media supplemented with 10% fetal bovine serum (Himedia, India), 50 µg/ml of antibiotic (Gentamycine) and stimulated by phytohaemagglutinin-M (Sigma-Aldrich, Inc., St. Louis, MI, USA) (1 µg/ml). Culture was incubated at 37 °C for 72 hours. Cultures was then treated with colchicine (Sigma-Aldrich, Inc., St. Louis, MI, USA) at a concentration of 0.02 µg/ml for 70 hours of culture to arrest the cells in metaphase. Cells were pelleted down by centrifugation at 1500 rpm for 5 minutes. The supernatant was discarded and the pellet was broken down. Cells were treated with pre-warmed hypotonic solution (appendix I) at 37ºC for 12 minutes. Cells were fixed with 4-5 drops of fixativemixed properly with pasture pipette and centrifuged at 1500 rpm for 5 minute. The supernatant was discarded, the pellet was broken to homogeneous suspension, suspended into fixative by adding drop by drop and finally maintained to 8 ml with fixative. The above step was repeated for 3-5 times until clear cell suspension was obtained. A drop of cell suspension was dropped on a clean, grease-free slide from 6 inches height and exposed to flame for instant drying. Slides were kept at 60ºC for overnight after preparation. For solid staining, slides were stained with 5% Giemsa stain (appendix I) for 5 minutes, air dried and mounted with DPX.

2.3. Karyotyping

55-65 metaphases were captured with the help of microscope and karyotyping was done with 450 G-banding resolution using Ikaros karyotyping system-Metasystems software (Carl Zeiss Microscopy Gmbh, Göttingen, Germany). Chromosomes were analysed following guidelines provided by the International System for Human Cytogenetic Nomenclature (ISCN 2013).

2.4. G-Banding of human chromosomes

For G-banding, 1 ml trypsin (appendix I) was mixed in 49 ml 0.9% NaCl in coupling jar and pH was adjusted to 7.5 by adding few drops of 1N NaOH. Slides were treated with trypsin solution for 8-20 seconds depending upon result to get good quality of banding. Immediately slides were transferred in a coupling jar containing 50 ml of phosphate buffer (appendix I). Slides were rinsed with distilled water in a separate coupling jar. Subsequently, slides were stained with 5% Giemsa for 5 minute and mounted with DPX.

2.5. Fluorescence in-situ hybridization (FISH)

2.5.1. Nick translation DNA labeling

Labeling of plasmid DNA clone was done using Nick translation kit (Enzo Life Sciences, Inc.) following manufacturer instruction. For 50 µl nick translation reaction, 1 µg of plasmid DNA was used. Reagents used in 50 µl reaction mixture of nick translation reaction were 5.0 µl of reaction buffer, 5.0 µl dNTPs mix, 2.5 µl of dTTP, 2.5 µl of Fluorophore-dUTP (0.3mM), 5.0 µl of freshly diluted DNase I (1:80 dilution), 5.0 µl of DNA polymerase I and rest nuclease free water. All the above reagents were mixed gently by flicking and centrifuged briefly. Reaction was incubated at 15 ºC for 2 hours. The reaction was terminated by adding 5 µl of stop buffer and incubated at 65ºC for 5 minutes. Placed on ice for 5 minutes prior to use and stored at 4ºC.

The slide was prepared by dropping one drop of fixative and one drop of cells suspension from height and passed through flame and then the slide was kept at 60ºC for overnight. Precipitation of nick translated probe was done for one reaction, 5.5 µl of nick translation reaction was taken into an eppendorf tube. 1.6 µl of 3M Sodium acetate (pH 5.5) was added and the total volume 16 µl was maintained by adding 7.9 µl of nuclease free water. 40 µl of chilled absolute alcohol was added, mixed and incubated at -80ºC for 30 minutes. For precipitation, centrifugation was done at 12000 rpm for 30 minutes at 4ºC. The supernatant was carefully removed and the pellet was dried at 37ºC in the dark for 1 hour. The probe was then suspended in 3 µl of nuclease free water and 7 µl of hybridization buffer (deionized formamide + 1 X SSC).

The labeled probe was denatured by incubation at 73ºC in water bath for 5 minutes and placing the tube immediately on ice for 2 minutes. The denatured probe was mixed and prewarmed at 37ºC for 15 minutes before hybridization.

2.5.2. Hybridization of probe

Denaturation buffer was prewarmed (70% formamide, 2XSSC, pH 7.0-8.0) at 73ºC for 30 minutes and slide was immersed for 5 minutes. Serial dehydration was done in 70%, 85%, and 100% alcohol for 1 minute in each. Slide was placed on hot plate maintained at 45ºC until hybridization. 10 µl of denatured probe was applied on the slide and mounted with cover slip and sealed with DPX. Slide was incubated at 37ºC for 16 hours in a humified slide chamber (50% formamide, 2X SSC used to control humidity).

2.5.3. Post hybridization wash and counterstaining and mounting

The wash solution 1 was prewarmed at 73ºC for 10 minutes. The cover slip was removed and the slide was dipped in wash solution 1 for 2 minutes. The slide was transferred into wash solution 2 for 1 minute at room temperature. The slide was air dried in the dark. 10 µl of mounting medium containing DAPI and DABCO (anti-fade) in 1:1 ratio was used for mounting. Slides were visualized under fluorescent microscope using appropriate fluorescent filter sets.

2.5.4. Genomic DNA extraction form peripheral blood

Genomic DNA was extracted from peripheral blood samples by salting out method (Miller *et al.* 1988). About 2.5 ml heparinized blood was added with 10 ml of 0.9% NaCl. Incubated for 5 minutes at room temperature and centrifuged at 5000 rpm for 5 minutes. The supernatant was discarded without disturbing the pellet. The pellet was broken down properly and added with 10 ml of solution A (stored at 4ºC). Mixing was done by gentle shaking for 5 minutes followed by centrifugation at 5000 rpm for 5 minutes. The supernatant was discarded, added with 1 ml of solution B and mixed properly for 5 minutes. 0.125 ml of solution C (appendix II) was added and mixed properly. 1 ml of chilled chloroform was added and mixed for 5 minute. Centrifugation was done at 5000 rpm for 5 minutes. Supernatant was separated in to other sterilized tube and added with equal volume of isopropanol to precipitate the DNA. DNA was transferred to eppendorf tube and washed 2-3 times with 70% alcohol. The pellet was dried at 37ºC for 2-3 hours. Finally dried pellet was dissolved in about 150 µl of TE.

2.5.5. Cytogenetic microarray

Cytogenetic microarray experiment was performed using cytogenetic 2.7M array and Affymetrix microarray work station (Affymetrix, Inc. Santa Clara, CA, USA). The Cytogenetics 2.7M array provided unbiased, whole genome coverage with the density of 2.7 million markers to enable superior resolution. It also included 400,000 single nucleotide polymorphisms (SNPs) to enable the detection of loss of heterozygosity (LOH) and uniparental disomy (UPD). The Cytogenetics 2.7M array was used to detect known and novel chromosome aberrations across the entire genome.

Whole genome amplification with 99 ng of genomic DNA from each sample was performed using kit provided by Affymetrix (Affymetrix, Inc. Santa Clara, CA, USA). Purification of amplified genomic DNA was performed using cyto magnetic bead, cyto wash buffer and cyto elution buffer following the Affymetrix Cytogenetics Assay Protocol User Manual. Eluted DNA was checked for its optimum quality and quantity by OD260/OD280 ratio between 1.8-2.0 and DNA concentrations >0.55 µg/µl. Fragmentation and labeling was performed with about 2 µg of purified DNA using cyto fragmentation and labeling buffers and enzymes. Fragmentation reaction was checked by running a 3-4% agarose gel. DNA fragments ranging between 50bp to 150 bp was considered optimum fragmentation before proceeding to hybridization reaction. Cyto hybridization buffer was mixed with fragmented samples and denaturation was performed on thermocycler. After denaturation, sample was immediately loaded in arrays and checked for any bubble (if there it should be movable). Arrays in trays were rotated at 60 rpm in hybridization oven maintained at 50 °C for 16 to 19 hours. After completion of hybridization, arrays were subjected for washing and staining using GeneChip Fluidics Station 450. The standard bleaching protocol was run on the GeneChip Fluidics Station prior to using Fluidics Station for processing Cytogenetics Arrays. Bleaching, washing and staining were performed by GeneChip Fluidics Station 450 under control

of software AffymetrixGeneChip Command Console (AGCC). Washing and staining of hybridized arrays was performed using cyto stain buffer 1, cyto stain buffer 2, cyto holding buffer, cyto wash buffer A and cyto wash buffer B. After completion of washing and staining, arrays were scanned by GeneChip Scanner 3000 7G under control of software AGCC scan control. CEL files generated after scanning were run and analyzed in Chromosomal Analytical Suit (ChAS) software. For genomic duplication and deletion analysis filter was set at 400 kbp deletion and duplication standard.

2.6. Statistical analysis

Mean=+- SD (Standard deviations)

3. Results

3.1. Clinical features in DS cases

Clinical features of DS children were recorded according to clinical phenotype checklist and frequency of different clinical features in DS patients was calculated (**Table 1**). Wide spectrum variability in clinical phenotypes was observed in DS children. None of the clinical features were common to all DS children. The same clinical features had variable severity among the DS children. Some of the clinical features were quite common and some were rarely presented by the DS children.

The mean age of mothers of DS children was 27.5±5.6 years at the time of the DS child birth. The mean age of DS children and fathers included in the study was found to be 10±2 months and 31.5±6.6 years, respectively. Majority of the birth of DS children were observed at younger maternal age and most of the incidence of DS child birth was at first parity and subsequently it incidence was declining.

Table 1: Chromosomal subtypes of Down syndrome.

Categories	**Number of cases** **Cohorts=N (%)** **Total suspected=131**
1. Confirmed DS cases of trisomy 21	96=(73.28%)
2. Robertsonian translocation and Isochromosome 21 of DS	3=(2.29%)
3. Mosaic of DS	0=(0%)
4. Partial duplication of DS	0=(not yet scored)
5. Unusual DS	32=(24.42%)

3.2. Molecular clinical cytogenetic analysis of suspected DS cases

A total of 131 suspected cases of DS were registered in a time period 2008 to 2015. **Category 1.** Out 131 of these cases,96(73.28%) cases were karyotyped After karyotyping, cases of these had trisomy 21 while **Category 2,** 3(2.29%) showed cases with translocation or isochromosome involving chromosome 21. The chromosomally cases were confirmed by FISH using a pericentromeric plasmid probe pZ21A.

FISH was performed with probe of pericentromeric region of chromosome 21 (pZ21A) having homology with pericentromeric region of chromosome 13. Therefore, four signals on interphase and metaphase (two from each chromosome 21 and 13) of normal individual were generally observed. In case of DS individual five signals on interphase and metaphase with two signals on chromosome 13 and three on chromosome 21 were observed. In Robertsonian translocation cases, signal on submetacentric position was observed showing karyotype 46,XX,rob14;21 or 46,XY,rob14;21). In case of isochromosome 21, signal on metacentric position was observed showing karyotype 46,XX,i21;21 or 46,XY,i21;21.

Category 3, was 0 mosaics cases. **Category 4,** was 0 not yet scored is that case comprised karyotypically 46,/XY but show partial duplications of any or Down syndrome critical region (DSCR) on the long arm of the chromosome 21.

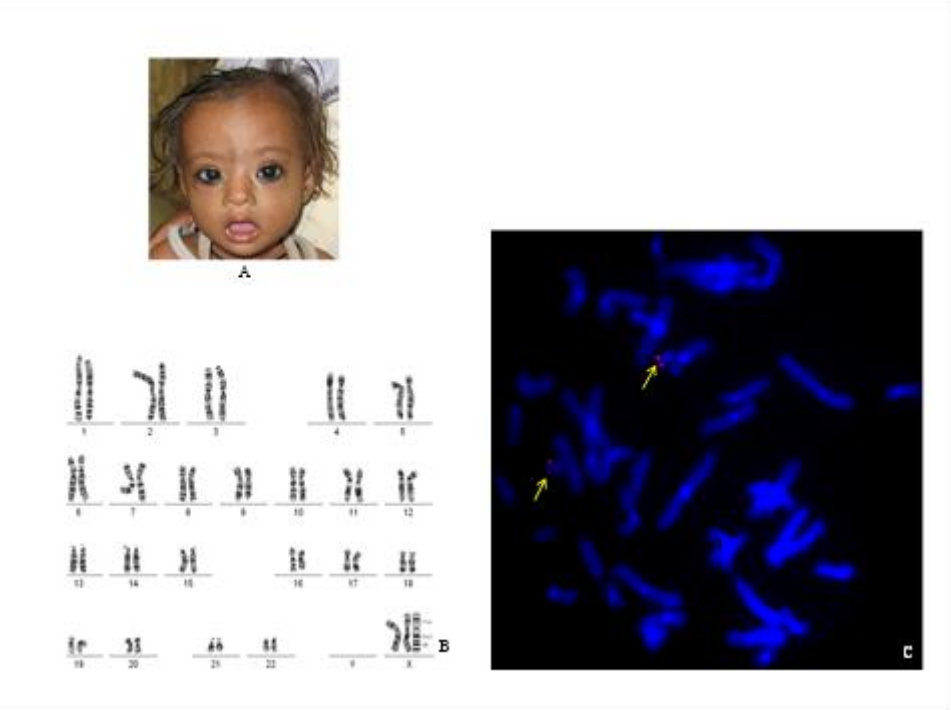

Figure 2 A-unusableDS case. A-a female child showing resemblance with Down syndrome features but, B-46,XX Karyotype. B-FISH hybridized by LS1 21 probe of DSCR region

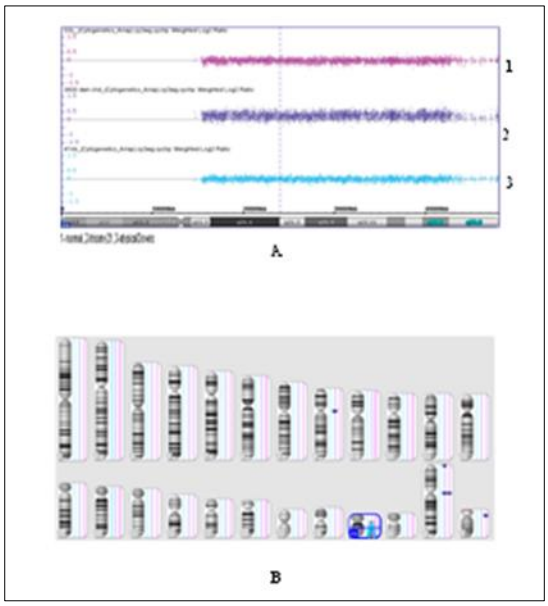

Figure 3 Oligo-based cytogenetic arrays. A-unusable female DS case as in figure 2, 1 is normal, 2 is trisomy 21 and unusable is 3. B-No duplication or deletion of any part of chromosome 21 or whole genome micro-duplication or deletion of region on chromosome 21 other than the DSCR.

The proposed unusual Down syndrome (**Figure 2 and 3**). is **Category 5,** had remaining 32(24.42%) cases, though referred for DS were karyopically 46,XX or 46,XY. Of these only one cases were judiciously selected and tested for partial duplication of Down syndrome critical region (DSCR) with the help of FISH by using LSI 21 chromosome 21 specific probe but as such no duplication was observed. One of the above an case was further investigated through oligo-based cytogenetic array for micro-duplication or deletion of region on chromosome 21 other than the DSCR and it revealed no duplication or deletion of any part of chromosome 21 or whole genome. The above study suggests that besides trisomy 21 and chromosomal rearrangements there might be genetic imbalances leading to perturbations of pathway/s which

require proof of evidence that could be responsible for expressed phenotype in these unusual Down syndromes. There is no such evidence till date, this is the first of it's own kind so, we are claiming that this a novel type of Down Syndrome.

4. Discussion

In the present study, blood samples taken from the children were used for chromosome analysis (Mitelman, 2013, Henegariu 2001) and DNA extraction. The results of the present study could be summarised as follows: Clinical and molecular cytogenetic analysis of Down syndrome allowed the identification of different categories.

4.1. Clinical phenotypic features present in DS children

More than 80 clinical features have been observed in DS including cognitive impairments, muscle hypotonia, short stature, facial dysmorphisms, congenital heart disease and several other congenital anomalies. DS children were presented with various combinations of clinical features, of which frequency and manifestation is also variable. For early diagnosis, knowledge of clinical features presented by DS children is important for clinician and health professionals. Late diagnosis may result in to delayed preliminary intervention of appropriate preventive measures for some risk conditions such as congenital heart defect, physical and psychological development which may otherwise increase morbidity and mortality. It has been considered that difference in clinical features presentation may reflect cytogenetic profile (proportion of free trisomy, translocation and mosaicism cases) of the DS children. Other factors contributing to clinical heterogeneity in DS children may be allelic heterogeneity for chromosomal genes present in three copies, individual's genetic make-up and environmental factors (Reeves, 2001).

4.2. Molecular clinical cytogenetic analysis of DS cases

DS is common chromosomal disorder. Its prevalence in India has been reported to be around 0.88 to 1.09 per 1000 live births (Verma, 2000). In Europe its prevalence is about 9.8 in 10 000 live birth (Dolk H *et al.* 2005), while in the USA it is 8.5 in 10 000 newborns in younger mothers (< 35 years) and up to 55.3:10000 newborns in advanced maternal aged mothers (>35 years) (Siffel*et al.* 2004). The present study documented DS cases referred from SS Hospital, Banaras Hindu University, Varanasi in a period of 2011-2015 from North India which included Eastern Uttar Pradesh and adjoining region of Bihar, Chhattisgarh and Jharkhand. The cytogenetic study was conducted on 131 suspected cases of DS. After kayotyping, 131 cases were cytogenetically confirmed to have trisomy of chromosome 21. Free trisomy 21 was significantly more frequent (95.6%) than the other types of trisomy 21, whereas mosaicism was observed in 0 % of the cases. In our registered cases of Robertsonian translocations, only the translocation of 14;21 was observed. Two cases of isochromosome 21 were also observed which is due to duplication of long arm of chromosome 21 and transverse splitting of the centromere.

Frequency of trisomy observed in the present study was higher than those reported from other part of the country like Verma *et al.* 1991 reported 93%, Chandra *et al.* 2010 reported 83.82% and Sheth et al, 2007 reported 84.8% whereas frequency of Robertsonian translocation and level of mosicism was lower in our study compared to report from these studies. Cytogenetic analysis of DS cases from India reported different frequency of trisomy ranging from 83.6%-97.8%. The frequency of translocation ranged from 2.2%-13.7% and mosaic karyotype was observed in range of 0-11.6%. Frequencies of different chromosomal subtypes in our registered DS cases were consistent with earlier reports. Slight differences among different reports might be due to the size of the samples. One of the several worldwide studies, Mutton *et al.* 1996 reported trisomy in 95% of the cases, Robertsonian translocation in 4% and mosaicism in 1% of the DS cases in England and Wales population. Similarly, Kava *et al.* 2004 observed free trisomy in 95%, translocation in 3.2% while mosaics were present in 1.8% of the DS cases. Overall, these discrepancies in the frequency of cytogenetic anomalies might be due to differences in the time period of analysis and population studied.

The proposed unusual Down syndrome (**Figure 2 and 3**). is **Category 5,** had remaining 32(24.42%) cases, though referred for DS were karyopically 46,XX or 46,XY. Of these only one cases were judiciously selected and tested for partial duplication of Down syndrome critical region (DSCR) with the help of FISH by using LSI 21 chromosome 21 specific probe but as such no duplication was observed. One of the above a case was further investigated through oligo-based cytogenetic array for micro-duplication or deletion of region on chromosome 21 other than the DSCR and it revealed no duplication or deletion of any part of chromosome 21 or whole genome. The above study suggests that besides trisomy 21 and chromosomal rearrangements there might be genetic imbalances leading to perturbations of pathway/s which require proof of evidence that could be responsible for expressed phenotype in these unusual Down syndromes. There is no such evidence till date, this is the first of it's own kind so, we are claiming that this a novel type of Down Syndrome

5. Conclusion

In brief, we had focused on certain of genetic aspects of Down syndrome in BHU, Varanasi. Overall frequencies of free trisomy, translocation and mosaic appear to be as with the global data (Jyothy et al., 2001). Our preliminary data on young DS mothers are particularly encouraging, and need to be extended on different populations in this country. It was discovered that after karyotyping, each of genetic disorders are of several sub-types, like in we have now DS cases, subtype is, free trisomy 21, isochromosome 21, translocation involving chromosome 21, duplication of some or all portion of chromosome 21. Clinical correlation was studied between different phenotypes of all sub-types of DS. This study suggested besides trisomy 21 and chromosomal rearrangements there might be genetic imbalances leading to perturbations of pathway/s could result unusual Down syndrome.

Compliance with ethical standards

Acknowledgments

We thank child and guardians for giving consent to obtain blood sample and publish the data. We are also thankful to Centre for Genetic Disorders for chromosomal culture facility, Interdisciplinary School of Life Science for cytogenetic microarrays and BHU for internal financial support.

Disclosure of conflict of interest

No conflict of interest.

Statement of ethical approval

All the performances were approved by the Human Research Ethics Committee. (Dean/2012-13/196 Dated 29.08.2012). Children and guardians give consent to obtain blood sample and publish the data.

Authors' contributions

- SKJ: Designed this project, performed the experiments, collected the data and conceptualization.
- AKR: Fund arrangement, major inputs, data interpretation, correspondence and corrections in the manuscript.

Funding

This work was supported by Department of Biotechnology, New Delhi, India (BT/01/COE/05/11 dated 27th September,2006).

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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