

Cytogenetic Analysis of Down Syndrome Patients in Eastern Uttar Pradesh

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ABSTRACT

Introduction: Down syndrome (DS) or trisomy 21 is the most common type of chromosomal abnormalities in new-born. There are three types regular (Free) Trisomy 21, Translocation and Mosaic Trisomy 21. One third cases of Down syndrome, clinical diagnosis may not be confirmed. Therefore, in this study we aimed to confirm the suspected Down syndrome patients by a cytogenetic analysis and also evaluate the risk factors associated with Down syndrome.

Material and methods: Total 30 suspected Down syndrome patients with aged between days 2 to 20 years old were included in this study, on the basis of well-defined inclusion criteria. The cytogenetic analysis, karyotype was carried out for all 30 suspected patients from peripheral blood and staining with Giemsa (G-Banding).

Results: Total of 30 children were included in which 16 patients with Down syndrome and 14 with normal. Regular (Free) Trisomy 21 was found in 93.75% patients and translocation was seen in 6.25% case. Whereas Mosaic down syndrome was not seen in any cases. Among Down syndrome, 10 (62.50%) were males. The mean maternal age at birth was significantly higher (31.94±3.04 years) in Down syndrome

Conclusion: Our results suggest that regular trisomy 21 is more common in Down syndrome cases. Moreover, higher maternal age was the major risk factor for Down's syndrome.

Keywords: Cytogenetic Analysis; Down Syndrome; Karyotype; Maternal Age; Translocation

Whereas mosaic Down syndrome is develop by mitotic non-disjunction in a chromosomally normal zygote.⁸ The incidence of translocation was present in 4% of down syndrome patients.¹ The extra chromosome 21 translocated to other chromosomes or to the acrocentric chromosomes of D (Chromosome 13,14,15) and G group (Chromosome 21,22) in down syndrome.¹

Till date, the reason of the non-disjunction error is not known. But there is a definite connection between down syndrome and maternal age.⁴ The increased maternal age at birth was established as a major independent risk factors for Down syndrome.¹⁻⁹ The genetic tendency was a third independent risk factor for Down syndrome.^{10,11} Karyotype, cytogenetic analysis is the standard method to categorize the chromosomal variants of Down syndrome.⁴ In this study, we aim to describe the cytogenetic profile of suspected Down syndrome children in the eastern region of Uttar Pradesh, India. We also evaluate the risk factors such as maternal age at birth associated with Down syndrome.

MATERIAL AND METHODS

This prospective study was conducted in the department of Centre for Advance Research (Cytogenetics Lab) and department of Pediatrics, King George's Medical University (KGMU), Lucknow, India during a period of one years (July 2018- June 2019). Total thirty suspected Down syndrome patients with aged between day 2 to 20 years old were included in this study, on the basis of well-defined inclusion and exclusion criteria. Children and adult have mild, moderate or severe intellectual and developmental problems

INTRODUCTION

Down syndrome (DS) is the commonest autosomal chromosomal abnormality and is main genetic cause of mental impairment and malformation. Worldwide, the incidence of down syndrome range from 1 in 600 to 1 in 700 and in India it is 1 in 1250 in live born infants.^{1,4} Down syndrome is detectible at birth. First time Dr. Langdon Down (1828 – 1896) was describe the precise clinical features of Down syndrome children.⁵ Down syndrome children shows the face, physical and mental impairment etc.⁶ Though, the accurate diagnosis of Down syndrome may be challenging in trisomy 21.⁷ So, the chromosomal analysis (Karyotype) is needed to confirm the diagnosis.

Down syndrome patients may be caused by three different types of chromosomal abnormalities: trisomy 21, translocation or mosaicism trisomy 21.² The regular (free) trisomy 21 is commonest types of chromosomal abnormalities, present in approximate 95% cases and it is occurs due to non-disjunction during maternal meiosis.^{1,3,4}

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with serious heart defects, Gastrointestinal (GI) defects, Immune disorders, Sleep apnea, obesity etc. were included in this study.

Ethical approval was obtained from the Institutional Ethical Committee and informed written consent was obtained from all the participants. All socio-demographic, physical and clinical data such as intellectual, developmental problems, distinct facial features and complication (heart defects, Gastrointestinal (GI) defects, Immune disorders, Sleep apnea, obesity etc.) were obtained from patients' medical records.

Information on age, maternal and paternal age at child birth and family history of Down syndrome at presentation were documented using a questionnaire.

Sample Collection

Total 2 ml peripheral blood of suspected Down syndrome patients were collected in Sodium Heparin vial for karyotyping.

Culture and Harvesting

Total 0.5 ml of blood sample was culture in 5ml RPMI 1640 (gibco ready to used media) in a 15ml screw cap culture vials under aseptic precautions. Incubate the culture vials capped loosely in CO₂ incubator at 37 °C, 5% CO₂ inject and 84% humidity (Incubator values set) for 70 hours. After 70 hours, 30 µl colcemid solution (10 micro gram per ml) was added and incubate for 1 hour at 37 °C and centrifuge at 1000 rpm for 10 minutes and discard the supernatant. Add 5 ml of hypotonic KCl (0.075 M) solution (Vortex well 1.5-2 mins) and incubate at 37 °C for 30 minutes. Centrifuge at 1000 rpm for 10 minutes, discard the supernatant. Add 5 ml of fixative (3:1 methanol-acetic acid) to the pellet, and mix well (can kept overnight). Centrifuge, discard the supernatant and add 5 ml of fixative, kept at 4 °C for 30 minutes. Give 2 more washes with fixative. Centrifuge, pipette out supernatant leaving about 0.5 ml of fixative. Use cells in 0.5 ml fixative for slide preparation.

Slide Preparation

Drop the cell suspension using Pasteur pipette for the height about 2 feet on the cold wet slide and blow the slide with humid air (mouth). Heat dry the slide slowly at 56 °C on a heating plate. Age the slides at room temperature for 3 days.

Banding (Trypsin Digestion)

Depending on the ageing of the slide, dip the slides in trypsin solution for 3 to 5 seconds. After that dip the slides in cold normal saline to stop trypsin activity and the wash under tap water. Keep the slides in Giemsa solution for 5 to 7 minutes and wash the slides in tap water. After drying, mount the slides with coverslip using DPX solution.

Metaphase Analysis

Metaphase was examine under Microscope (Nikon Eclipse 90i) with the help of Genikon software (Fig. 1). In each case, 20-25 metaphases were examined and 3-5 cells were photographed and karyotyped. In case of mosaicism, 50 to 100 metaphases were scored. Karyotype description was done according to the international nomenclature guidelines (ISCN 2013) (International Standard Committee on Human Cytogenetics Nomenclature).¹⁵

STATISTICAL ANALYSIS

Data were analyzed using the software SPSS, version 17. Categorical variables were presented as the number and percentage, when the quantitative variables were presented as mean ± standard deviation. Student's T test was used for comparison of means. P ≤ 0.05 was considered statistically significant.

RESULT

The baseline characteristics of suspected children and maternal age at child birth were shown in Table 1. Values are expressed as mean, median, ±SD, minimum and maximum. Range of age among suspected children were between 1 and 240 months. Mean age of children was 55.53±64.43 months. Range of maternal age at birth were between 21 and 39 years. Mean maternal age was 29.57±4.14 months. The suspected Down syndrome children was more common (56.7%) in male. Out of 30, total 22 (73.3%) children were from Hindu religion whereas 8 (26.7%) children were from Muslim religion. Out of 30 suspected children, 16 patients had Down syndrome and 14 patients had normal.

In this study, total of 30 children were included in which 16 patients with Down syndrome and 14 with normal [Table 2]. Total 10 (62.50%) were males and 6 (37.50%) were females in Down syndrome whereas 7 (50.0%) male and female in normal. The mean age at referral was

Parameter	Mean	Median	Std. Deviation	Minimum	Maximum
Age (months)	55.53	39.00	64.43	1.00	240.0
Maternal Age (years)	29.57	30.00	4.14	21.00	39.00
Sex	n	%	-	-	-
Male	17	56.7	-	-	-
Female	13	43.3	-	-	-
Religion			-	-	-
Hindu	22	73.3	-	-	-
Muslim	8	26.7	-	-	-
Trisomy 21					
Yes	16	53.33	-	-	-
No	14	46.67	-	-	-

Table-1: Baseline characteristics of children

	Trisomy 21 (n=16)		Normal (n=14)		p-Value
	Mean	±SD	Mean	±SD	
Age (months)	55.69	55.89	55.36	75.21	0.989
Maternal Age (years)	31.94	3.04	26.86	3.57	<0.001
Sex	n	%	n	%	
Male	10	62.5	7	50.0	0.749
Female	6	37.5	7	50.0	
Religion					
Hindu	13	81.25	9	64.29	0.526
Muslim	3	18.75	5	35.71	

Table-2: Comparison of baseline characteristics between trisomy 21 and normal children

Karyotype	No	%
Regular trisomy 21		
47,XY,+21	9	56.25
47,XX,+21	6	37.50
Translocation DS		
46, XY, +21, rob (21; 21) (q10; q10)	1	6.25
Mosaic DS		
47,XY,+21/46,XY	0	0.0
Total	16	100

Table-3: Distribution of Trisomy 21

Author	Total No	Regular trisomy 21	Translocation DS	Mosaic DS
Current	16	15	1	0
Das et al., 2015 ³	32	29(90.63%)	1 (3.13%)	2(6.25%)
Poddar et al., 2012 ¹²	45	42 (93.33%)	-	3 (6.67%)
Mandava et al., 2010 ¹³	1572	1400 (89.06%)	111 (7.06%)	29 (1.84%)
Chandra et al., 2010 ¹⁴	1020	855 (83.82%)	51 (5.0%)	110 (10.78%)
Jayalakshamma et al., 2010 ¹	870	756 (86.90%)	77 (8.85%)	37 (4.25%)
Verma et al., 1991 ¹⁵	2410	2207 (91.58%)	98 (4.07%)	98 (4.07%)

Table 4: Karyotype frequencies among studied Down syndrome cases from other India surveys

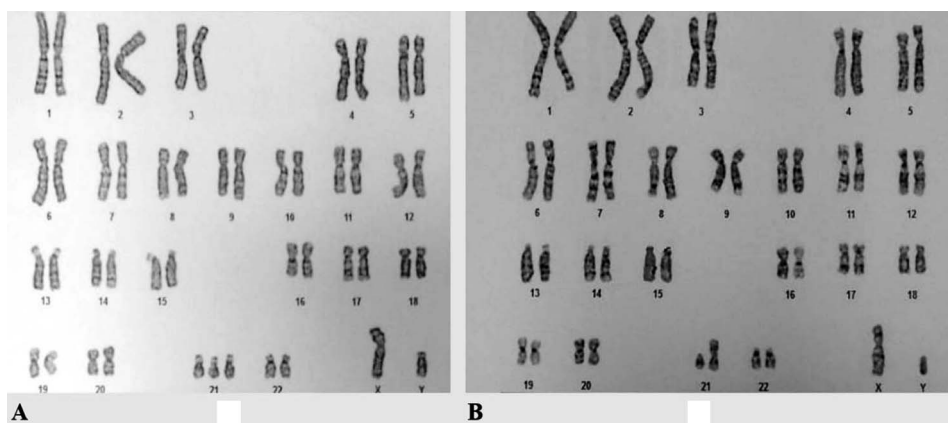


Figure-1: Karyotype showing [A] Free Trisomy 21; [B] Translocation Trisomy 21

55.69±55.89 and 55.36±75.21 months in Down syndrome and normal, respectively. The mean maternal age at birth was significantly higher (31.94±3.04 years) in Trisomy 21 as compared to normal (26.86±3.57 years) ($p<0.001$) in our studied population. About 81.25% patient were Hindu religion in Trisomy 21 whereas 64.29% were Hindu in normal.

The chromosomal analysis by karyotyping were undertaken in 30 suspected patients cases, out of which 16 had down syndrome among them 15 (93.75%) cases had free

trisomy 21, and 1 case had translocation (46, XY,+21, rob (21; 21) (q10; q10) with trisomy 21 [Table 3 and Fig. 1].

DISCUSSION

Down syndrome (Trisomy) 21 is a common birth defect. On the basis of clinical features, it can be easily diagnosed. Though, the conventional karyotyping is essential for the confirmation of Down syndrome (trisomy 21, mosaicism and translocation).

In our study, the overall sex ratio was 1.67:1. The Down syndrome was more common in male. Similarly, various studies reported that the males are more common in Down syndrome. Our results are similar to those found by Kolgeci et al. (2013) in Kosovo (1.72:1) and near to those of Amayreh et al. (2012) in Jordan (1.61:1).^{16,17} Moreover, Belmokhtar et al. (2016) reported that the overall male: female ratio was 1.75:1.⁴ The greater male sex ratio may be due to the inherent predisposition of Y chromosome associated to the group G chromosome to be nearer to its other members, 21 and 22, particularly, chromosome 21 is smallest acrocentric. The causes of more common male in Down syndrome related to the paternal errors are not clearly known till now.¹⁸

Though a great amount of evidence is available on numerous aspects of Down syndrome, a complete understanding of the fundamental mechanism(s) is yet to be established. In this study, the mean maternal age at birth was significantly associated with down syndrome. Similarly, Belmokhtar et al. (2016), Chandra et al. (2010), Qahatani et al. (2011), Jaouad et al. (2010), Mutton et al. (1996) and Verma et al. (1990) reported that the mean maternal age is higher in free trisomy 21 of Down syndrome.^{4,14,19-22} This effect may be due to differential choice and accumulation of trisomy 21 oocytes in the ovarian reserve of older women.²³

In our study, on the basis of clinical feature, total 30 cases were referred from different department of our University. Out of these 30 cases, 16 Down syndrome cases were identified. Total 93.75% children with Down syndrome have extra chromosome 21 and 6.25% children with Down syndrome have translocation. Not a single case of mosaicism was observed. Our study was supported by various Indian studies, they reported that the regular trisomy 21 is more common birth defect in Down syndrome (Table 4). The frequency of regular trisomy 21 in previous international studies in North Africa countries such as Algeria, Morocco, England and Wales, Egypt, Saudi Arabia, Turkey, China, France and Australia ranged from 91%-96%.^{4,20,21,24-29} Previously, various studies reported that the frequency of translocation was varied from 0.67% to 8.8% in Down syndrome, the lowest frequency was reported in UAE, Iran, and Malaysia and whereas the highest frequency was reported in India (8.8%).³⁰⁻³²

CONCLUSION

In this study we perform a karyotyping cytogenetic analysis for all clinically suspected Down syndrome patients to confirm the clinical diagnosis. We evaluate the frequency of different types of Down syndrome in our population. Our results suggest that regular trisomy 21 is more common in Down syndrome cases. These results were similar to many national and international studies. Moreover, higher maternal age was the major risk factor for Down's syndrome.

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