

## Original Research Article

# Association of trisomy 21 with anorectal malformation: a cytogenetic study

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### ABSTRACT

**Background:** Anorectal malformations (ARM) are congenital malformations of digestive system resulting from the disturbed development of hindgut during embryogenesis. ARMs involve both sexes; can occur either isolated or in association with other congenital abnormalities and may be associated with chromosomal abnormalities. Association of trisomy 21 with anorectal malformation is well documented. Present study was conducted to assess prevalence of association of trisomy 21 in patients with anorectal malformation.

**Methods:** Total 48 children with diagnosis of anorectal malformation, who were admitted in Department of Paediatric Surgery, King George's Medical University, Lucknow, Uttar Pradesh, were selected for the study. Blood samples were collected and their cytogenetic analysis was carried out in the Cytogenetics laboratory, Department of Anatomy, KGMU-U.P, Lucknow, India.

**Results:** Among the 48 study subjects, karyogram could be successfully obtained for 45 cases (93.75%). Numerical anomalies were observed in 8.9% cases. Trisomy 21 was found in 6.7%. The prevalence of trisomy 21 was found to more in males (4.5%) as compared to females (2.2%). Prevalence was highest in birth order 3(20%), followed by birth order 2(7.14%) and lowest in birth order 1(3.85%). Trisomy in association with ARM; was observed in children born to females aged >30 years. It was found unrelated to the history of consanguinity.

**Conclusions:** Prevalence of association of trisomy 21 with ARM was found to be 6.7%. This coexistence emphasizes the need for a thorough investigation of patients with ARM.

**Keywords:** Anorectal, Cytogenetic analysis, Down syndrome, Hindgut, Karyotyping, Trisomy

### INTRODUCTION

Anorectal malformations (ARMs) are among the frequently encountered congenital anomalies of hindgut development encountered in paediatric surgery. Estimated incidence has been reported as 1 in 2500 live births.<sup>1</sup> Anorectal malformations include a series of defects ranging from slight malposition of anus to more complex anomalies in development of the hindgut and urogenital organs.<sup>2</sup> Antenatal diagnosis of isolated ARM is rare. Most cases are diagnosed in the early neonatal period. ARM is multifactorial, chromosomal abnormalities are present in 4.5-11% of the patients with

ARM.<sup>3</sup> Several monogenetic syndromes have ARM as one of the possible features and already point to the involvement of specific genes in hindgut development. The association of Down syndrome with ARM is well reported. Several studies have reported association of Down with ARM; some authors have reported trisomy 21 in 2-5% of the patients with ARM, a study of 1,846 babies with ARM, observed chromosomal anomalies in 11%.<sup>4</sup> The frequency of trisomy 21 among babies with ARM has been reported to be 15 times higher, in comparison to neonates in the general population.<sup>5</sup> Changing social dynamics in the form of increase in prevalence of late marriages, the reproductive pattern of

society is changing drastically. Consequentially conception at an advanced maternal age poses a very high risk and may result in the birth of a child having chromosomal trisomy and syndromes which may result into an increased incidence of these disorders.<sup>6</sup> Many new chromosomal anomalies have been detected due to cytogenetic analysis techniques; hence the need of cytogenetic screening in recent perspective.

## METHODS

Study was conducted by the Department of Anatomy, King George's Medical University Lucknow, Uttar Pradesh, in collaboration with the Department of Paediatric Surgery, King George's Medical University Lucknow, Uttar Pradesh.

Ethical clearance was taken from ethical clearance board of King George's Medical University, Lucknow, Uttar Pradesh, vide letter number 88<sup>th</sup> ECM IIB- Thesis/P14 dated. Period of study was September 2017-September 2018.

Patients were screened in the Department of Paediatric Surgery and children diagnosed with anorectal malformations were shortlisted. 48 children (33 males, 15 females) were randomly selected for the study from this group after they satisfied the inclusion and exclusion criteria.

### Inclusion criteria

- Infants/ neonates clinically diagnosed as a case of anorectal malformations by the paediatrician.
- Patients willing to participate in the study.

### Exclusion criteria

- Subjects who were unwilling to participate.

Maternal history was taken regarding age, parity, previous deliveries, drug intake, acute and chronic illness during pregnancy, and any radiation exposure during pregnancy. Family history was taken for similar cases, chromosomal abnormalities and still births. Peripheral blood sample was used to prepare the karyograms.

Peripheral venous blood of the patient was collected in BD Vacutainer sodium heparin vial, taking due aseptic precautions, and further processing of the sample was done in the cytogenetics laboratory of the Department of Anatomy, King George's Medical University, Lucknow, Uttar Pradesh.

At which 0.5 ml of blood sample was added to 5ml of culture media in a test tube, under laminar air flow and the culture tube was incubated at 37°C temperature, 85% humidity and 5% concentration of CO<sub>2</sub> for 72 hours in slanting position in CO<sub>2</sub> incubator (YORCO). After incubation 5 drops of karyoMAX colcemid solution (0.1

microgram/ml) was added, and test tube was centrifuged at 1000 rpm for 10 minutes. The supernatant was discarded by pipetting of media, the cell button was suspended in 5 ml of hypotonic solution and was incubated at 37°C for 30 minutes. Centrifugation was done at 1000 rpm for 10 minutes; the cell button was re-suspended in 5 ml of fixative. This process of centrifugation, discarding the supernatant and adding the fixative was repeated 2-3 times until button at the bottom of the test tube became white. Finally the tube was kept at 2-3°C for 2 hours before the harvested blood cells became ready for slide preparation.

Slides were prepared by dropping method, and were treated with trypsin to obtain better banding. Adequately aged slides were stained with Giemsa stain. These slides were observed under microscope (Olympus BX51) attached with a computer and fields showing a good spread of metaphase were photographed. Karyograms were prepared from slides using Cytovision software and were analysed.

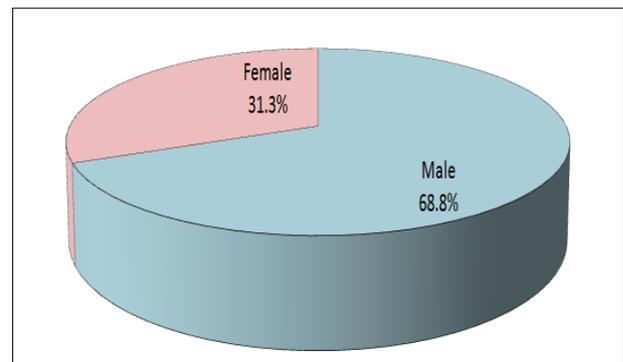
Karyotyping results were obtained by analysing 20 metaphase fields for each case and in cases where abnormal karyotypes were suspected, the observation was extended to a total of 30 fields. The karyotypes were reported as per international system for human cytogenetic nomenclature (ISCN, 2013) guidelines.

### Statistical analysis

The statistical analysis was done using SPSS (Statistical Package for Social Sciences) Version 21.0 statistical Analysis Software. Values were represented in numbers and percentages (%). Appropriate statistical formulae and tests were used for comparison of data.

## RESULTS

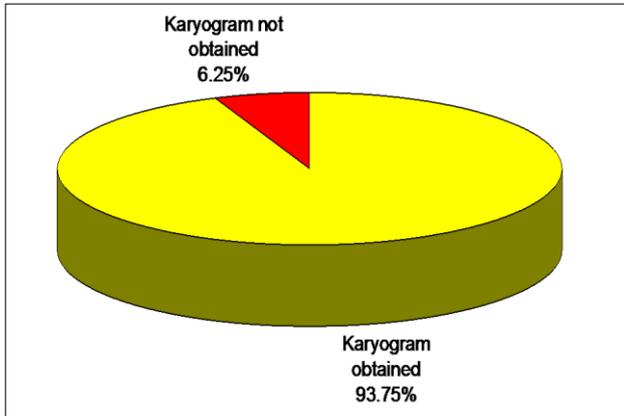
Among the 48 study subjects, majority of children were males (n=33; 68.8%); there were 15 females (31.2%) (Figure 1).



**Figure 1: Gender wise distribution of study subjects.**

Sex ratio (M: F) was 2.2. Birth order of the children ranged from birth order 1 to 3. Age of mothers ranged

between 19 to 36 years with a mean age of 25.04±4.10 years. Most of the mothers (91.7%) were ≤30 years of age. 39 cases had isolated ARMs whereas associated anomalies were present in only 9 cases. Consanguinity was reported in 10 cases. All the 48 samples collected were subjected to cytogenetic processing. Culture was successful in 45 out of 48 samples (93.75%), remaining 3 samples failed (Figure 2).

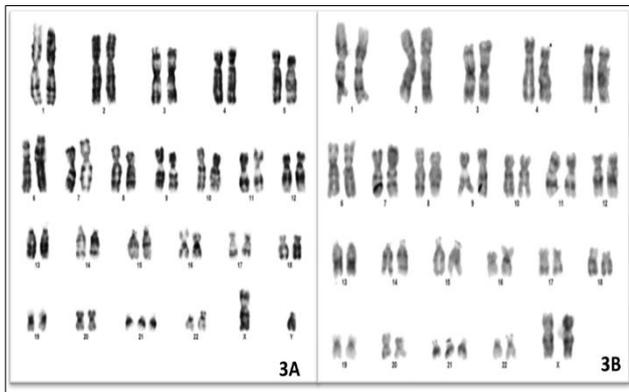


**Figure 2: Distribution of cases according to success in karyotyping**

Chromosomal anomalies were observed and reported in 4 cases out of 45 cases (Table 1). Trisomy 21 was reported in 3 cases out of 45 cases (6.7%); 2 cases were males whereas one was a female (Figure 3A and 3B).

**Table 1: Distribution of different types of karyograms obtained.**

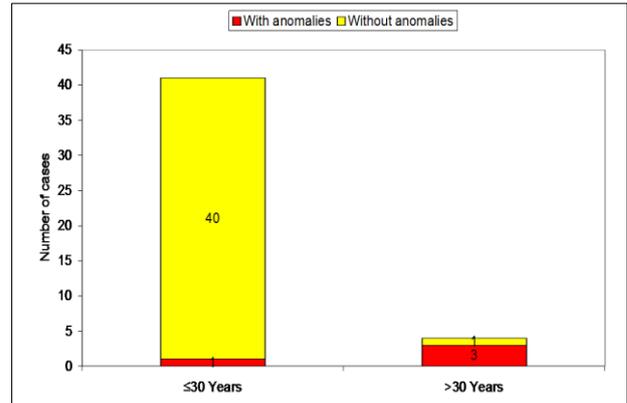
Outcome	No.	Percentage
Normal karyotypes	41	91.1
Numerical anomalies	4	8.9
Structural anomalies	0	0



**Figures 3: Karyotypes with Trisomy 21 in (3A) male and (3B) female.**

Out of 45 cases 26 were birth order 1, 14 were birth order 2 and 5 were birth order 3. Among the 3 cases displaying Trisomy 21, 1 child was birth order 1, 1 was birth order 2

and 1 was birth order 3. Prevalence of trisomy increased with increase in birth order. It was highest in birth order 3 (20%), followed by birth order 2(7.14%) and lowest in birth order 1(3.85%). In the study group there were only 4(8.9%) women who were ≥30 years of age at the time of birth of the child. Trisomy 21 was observed in 3 children; all 3 were born to mothers with age >30 years (Figure 4). No chromosomal anomalies were observed in the children, where history of consanguinity was positive.



**Figure 4: Correlation of maternal age with prevalence of chromosomal anomalies.**

**DISCUSSION**

Anorectal malformations are congenital malformations of digestive system resulting from disturbed development of the hindgut during embryogenesis. Chromosomal aberrations are disruptions in the normal chromosomal content of a cell and are a major cause of genetic conditions in humans. Some chromosome abnormalities such as translocations, or chromosomal inversions do not cause disease in carriers, although, they may lead to a higher chance of bearing a child with a chromosome disorder.

Mirza et al, conducted a study among 100 cases with ARM at Lahore Pakistan, out of which 77% were males and 23% (3.4:1).<sup>7</sup> Bălănescu et al reviewed 50 cases of ARM 28 were males (M) and 22 females (F).<sup>8</sup> In the present study, out of 48 cases, 33(68.7%) patients were males and 15(31.3%) were females, sex ratio M: F was 2.2. Verma et al, reported, that the babies with birth order 4 had significantly higher incidence of malformation ( $\chi^2 = 4.67, p < 0.05$ ).<sup>9</sup> Gupta et al in a study on 24 patients of anorectal malformations; reported 50%(12/24) children were birth order 1, 37.5%(9/24) were birth order 2 while the remaining 12.5% (3/24) were either 3<sup>rd</sup> or 4<sup>th</sup> in birth order.<sup>10</sup> The cytogenetic profile of Down syndrome includes free trisomy 21, Robertsonian translocations, mosaicism, duplication of the DS critical region and other structural rearrangements involving chromosome 21.<sup>11-13</sup> Most common karyotype encountered in Down’s syndrome children is of free Trisomy 21, while their parents have normal karyotype.

Endo et al reported trisomy incidence of 5.1% with no gender variations in a study population of 1992 ARM patients in Japan.<sup>14</sup> Reciprocally in different smaller series of patients with Down syndrome, ARM was present in 0.3-2% as reported by Bianca and Ettore.<sup>15</sup> Cho et al reported the frequency of trisomy 21 in 103 cases of anorectal malformation to be nearly 2%.<sup>4</sup> Gupta et al reported the incidence of trisomy 21 to be 17% in the 24 cases of anorectal malformation taken up for their chromosomal study done jointly in states of J&K and Haryana.<sup>16</sup>

In the present study, out of 48 cases of ARM, culture could be successfully obtained for 45 cases. Out of 45 cases of ARM 26 cases (57.8%) were of 1<sup>st</sup> birth order and remaining 19 cases (42.2%) were birth order 2-3. Among those 45 cases chromosomal anomalies were detected on the basis of karyotyping in 4 cases. Out of 4 cases with chromosomal anomalies; Trisomy 21 was observed and reported in 3 cases (2 cases were males whereas one was female). Among the 3 cases displaying Trisomy 21, 1 child was birth order 1, 1 was birth order 2 and 1 was birth order 3. Prevalence of trisomy increased with increase in birth order. It was highest in birth order 3 (20%), followed by birth order 2(7.14%) and lowest in birth order 1(3.85%). In the present study all the 3 cases of ARM with Trisomy 21 were born to mothers with age >30 years.

## CONCLUSION

Anorectal malformations (ARMs) are among the frequently encountered congenital anomalies of hindgut development seen in paediatric surgery. Chromosomal anomalies were co-associated with ARM and found in 8.8% cases. Commonest associated anomaly was Down's syndrome (6.66% cases). It was more commonly found in male cases (4.44%) as compared to females (2.22%). Advanced maternal age (mothers with age >30 years) was a common associated factor in all 3 children with trisomy 21.

Presence of trisomy in patients with ARM emphasizes the need for thorough screening for chromosomal anomalies. Identification of such associations will help to prevent future complications which are spectrum of Down syndrome and they could be managed in time.

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