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Original Research Article

Karyotyping to Assess Structural and Numerical Chromosomal Anomalies in Children with Anorectal Malformations

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Abstract

Anorectal malformations are one of the common congenital anomalies with an incidence of 1-3000 to 5000. Up to 70% of the patients have associated anomalies. Forty to seventy percent of ARM patients have one or more additional defects of other organ systems. The etiology of ARM is multifactorial, it includes both genetic and environmental. This study was done to determine the structural and numerical chromosomal associated with anorectal malformations. 150 patients were included in the study. A full physical examination of the child was conducted followed by an infantogram, echocardiogram, and spinal ultrasound scan was done to investigate different associated anomalies. The patients were classified according to the Krickenbeck classification. Karyotyping was done to determine the structural and numerical chromosomal anomalies. ARM with perineal fistula was the common type of ARM. Out of 150 karyotypes done, only two patients had abnormal karyotypes. Both patients had Downs syndrome. Consanguinity was found in 22% of the cases. Karyotyping plays a vital role in the comprehensive evaluation of patients with anorectal malformations by identifying chromosomal abnormalities, guiding management decisions, providing prognostic information, and enabling genetic counseling.

Keywords: Anorectal malformation, karyotype, Downs syndrome, consanguinity.

INTRODUCTION

Anorectal malformations (ARM) are one of the common congenital anomalies affecting the hindgut. ARM has an incidence of 1in 2000 to 1 in 5000 live births [1]. ARM includes a variety of malformations ranging from mispositioning of anal opening to complex anomalies involving hindgut and urogenital organs [2]. Forty to seventy percent of ARM patients have one or more additional defects of other organ systems [3]. The etiology of ARM is multifactorial, it includes both genetic and environmental. 4.5% - 11% of patients with ARM will have chromosomal anomalies [4]. Chromosomal anomalies associated with ARM include Trisomy 8 mosaicism, trisomy 13, trisomy 18, trisomy 21, trisomy 22, sex chromosome aneuploidy, triploidy, tetrasomy 12p, ring 13, deletion 5q etc. [5].

MATERIALS AND METHOD

This study was conducted in the Department of Paediatric Surgery, Bangalore Medical College and Research Institute from Jan 2014 to Dec 2016. All children with ARM treated and followed up in the department were included in the study. Written informed consent was obtained from the parents of the patients. The study was cleared by the institutional ethical committee.

A blood sample of about 2-3ml was collected in a sodium heparin vacutainer. Cell culturing was done in a sterile room that had a laminar airflow. Working media was prepared using plain media- RPMI1640, Fetal bovine serum (FBS), Glutamine, and Phytohaemagglutinin (PHA). Then 720 µl blood was added to the working media in a tube and incubated for 72 hrs at 37ºC in a CO2 incubator. After 72 hrs 60 µl of colchicine was added and then incubated for one hour. After one hour, the tube is centrifuged and hypotonic

treatment is done by adding KCl and keeping it in a water bath for 10 mins at 37ºC. Then 1ml of the prefix is added (fixative- 1:3, acetic acid: methanol) and centrifuged. This is repeated till the supernatant becomes clear. Then the solution is dropped onto a clean slide rinsed with fixative and chilled in the refrigerator. then the slide is baked in the hot air oven for 45 minutes at 90 C. Then the slides are stained using Giemsa stain. Slides are checked under low power and then under 100x m for good spreads, trypsinisation, and staining and are analyzed using the applied spectral imaging software.

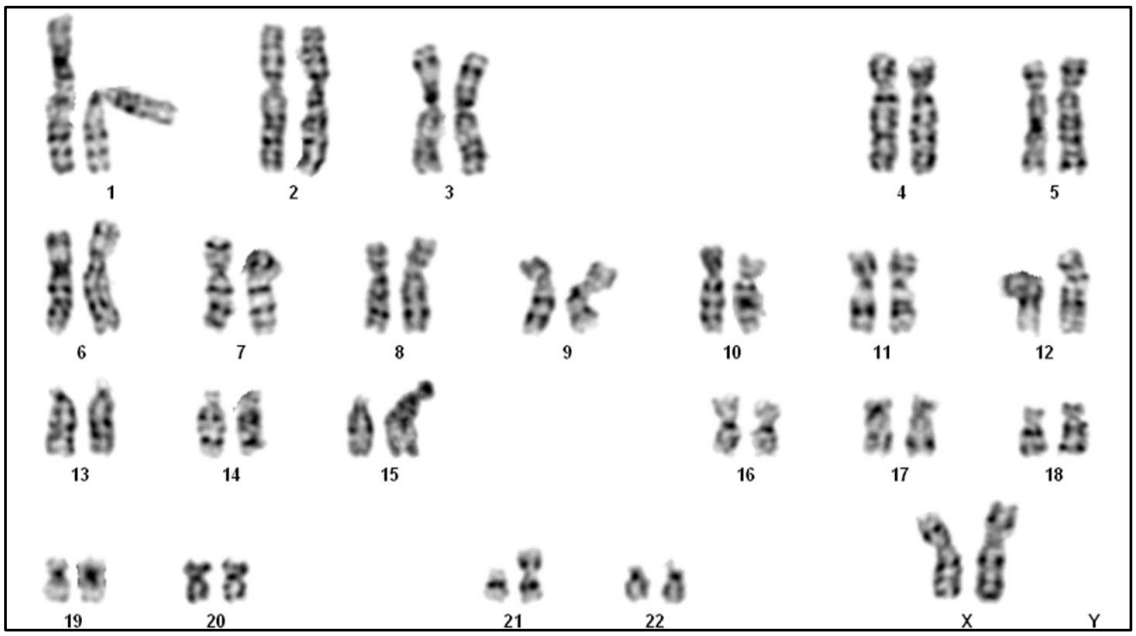
RESULTS

150 patients were included in the study. These patients were classified according to the Krickenbeck classification. 101 (67.3%) were males and 49 (32.7%) were females. Perineal fistula (20%) was the most common type of anomaly present in our study. Vestibular fistula was the frequent anomaly in females while in males it was rectobulbar fistula and no fistula. Associated anomalies were present in 111 (74%) patients while 39 (26%) had isolated ARM.

Table 1: Type of ARM: Krickenbeck classification

Type of ARM	Number (%)	Male	Female
Cloaca	7 (4.7)	NA	7
No Fistula	28 (18.7)	28	0
Rectobulbar Fistula	28 (18.7)	28	NA
Rectoprostatic Fistula	21 (14.0)	21	NA
Vestibular Fistula	22 (14.7)	NA	22
Pouch Colon	6 (4.0)	4	2
Rectal Atresia	4 (2.7)	3	1
Rectovaginal Fistula	2 (1.3)	NA	2
Anorectal Agenesis	2 (1.3)	0	2
Perineal Fistula	30 (20.0)	17	13
Total	**150**	**101 (67.3%)**	**49 (32.7%)**

Out of 150 karyotypes done, only two patients had abnormal karyotypes. Both patients had Downs syndrome. One patient had 46,XX,der (21;21)(q10;q10),+21 (Down's syndrome – translocation type). This patient had rectovaginal fistula. Other associated anomalies found in this patient were ASD, Coarctation of the aorta, and PDA. The maternal age of this patient was 45 years. The other patients with Downs syndrome were ARM with no fistula type along with ASD and PDA.



46, XX, der (21; 21) (q10; q10), + 21 (Down's syndrome – translocation type)

Consanguinity was found in 22% of the cases. 36.7%(P<.05) of the patients with perineal fistula were born to consanguineous couples. All the patients with cloaca were born to non-consanguineous couples.

Table 2: ARM classification and consanguinity

Consanguinity	Cloaca	No fistula	Others	Perineal fistula	Pouch Colon	Rectal atresia/ Stenosis	Recto bulbar fistula	Rectoprostatic fistula	Rectovaginal Fistula	Vestibular fistula	Total
Absent (%)	7 (100)	22 (78.5)	2 (100)	19 (63.3)	6 (100)	3 (75)	25 (89.3)	15 (71.4)	2 (100)	16 (72.7)	117 (78)
Present (%)	0	6 (21.4)	0	11 (36.7)	0	1 (25)	3 (10.7)	6 (28.6)	0	6 (27.3)	33 (22)

The mean maternal age was 24.04±4. 95.3% of the mothers were between the age of 21-35 years. Only one mother was above the age of 40 and the baby of this mother had rectovaginal fistula and Downs syndrome.

Table 3: ARM classification and maternal Age

Maternal Age	Cloaca	No fistula	Others	Perineal fistula	Pouch Colon	Rectal atresia/ Stenosis	Recto bulbar fistula	Rectoprostatic fistula	Rectovaginal Fistula	Vestibular fistula	Total
<20	0	1	0	1	1	1	1	1	0	0	6(4%)
21-35	7	27	2	29	5	3	27	20	1	22	143(95.3%)
36-40	0	0	0	0	0	0	0	0	0	0	0
>40	0	0	0	0	0	0	0	0	1	0	1(0.7%)

The mean paternal age was 29.34±4.4. 90.7% of fathers were in the age group between 21-35 years. 9.3% were in the age group between 36-45 years.

Table 4: ARM classification and paternal Age

Paternal Age	Cloaca	No fistula	Others	Perineal fistula	Pouch Colon	Rectal atresia/ Stenosis	Recto bulbar fistula	Rectoprostatic fistula	Rectovaginal Fistula	Vestibular fistula	Total
<20	0	0	0	0	0	0	0	0	0	0	0
21-35	4	28	2	29	6	4	24	19	1	19	136(90.7%)
36-40	3	0	0	1	0	0	4	2	1	3	14(9.3%)

4 (2.6%) had a family history of anorectal malformation. 2 kids from the same family were affected by anorectal malformation. There was no consanguinity in the parents.

50% of the patients were the first child. 37.3% were second in the order of birth.

Table 5: Order of birth

Order of Birth	No (%)
1St	75(50%)
2nd	56(37.3%)
3rd	15(10%)
4th	3(2%)
5th	1(0.67%)

DISCUSSION

Anorectal malformations are frequently encountered congenital anomalies posing challenges to pediatric surgeons. ARM has an incidence ranging between 1 in 1500 to 1 in 5000 [6, 7]. The presence of associated anomalies is one of the key factors that determine morbidity, mortality, and long-term functional outcomes. The gender distribution associated with ARM is almost equal [6, 2]. But in a few variants of ARM male and female preponderance is seen [5]. In the present study, the male-female ratio was 2:1. Rectobulbar fistula and ARM with no fistula were the most common types of ARM present in the males, while in females vestibular fistula was common followed by ARM with perineal fistula. Balanenscu *et al.,* [2] reported the presence of 22% of patients with vestibular fistula and 40% with no fistula. Rollins [8] reported the occurrence of perineal fistula in 40.4% of males and vestibular fistula in 27.6% which was almost similar to the findings in our study.

ARM is a multifactorial disorder that involves environmental or genetic risk factors which are still largely unknown. Several monogenetic syndromes have an ARM as one of the possible features and point to the involvement of specific genes in hindgut development [4]. Chromosome abnormalities are present in 4.5–11% of the patients with ARM [9, 10]. In our study, only 2(1.3%) out of 150 patients had chromosomal abnormalities. Both patients had Downs syndrome. The association of Down syndrome with ARM is found in about two to five percent of the patients [11-13]. They constitute about 40% of the patients with an ARM and a chromosomal anomaly. In different smaller series of patients with Down syndrome an ARM was present in 0.3–2% [14, 15]. Over 95% of patients with Down syndrome and an ARM have an imperforate anus without fistula [7, 16, 17].

Hassink *et al.,* [18] did a study between 1974 and 1995 to identify additional congenital defects in patients with anorectal malformation. Out of 264 patients, nine had chromosomal abnormality (3.4%) and 5 of these had a trisomy 21. The association of terminal deletions of the long arm of chromosome 7 with ARM is well known and multiple cases have been described [19-21].

Anorectal malformations have been identified along with Pallister–Killian syndrome (PKS) [22, 23]. The syndrome characterized by deletion in chromosome 13q is most frequently linked with anorectal malformations [23]. Allderdice *et al.,* [24] have reported 13q-deletion syndrome in 5 out of 23 cases that he had studied. Trisomy 16 is one of the most common autosomal trisomies in spontaneous abortions. Anorectal malformations have been reported in several cases with non-mosaic trisomy 16 [25].

Older maternal age is associated with chromosomal and genetic risk. The risk of complex anorectal malformations is 31% higher for children of mothers aged ≥35 years and 13% higher for children of mothers aged ≤24 years compared with ages 25–34 years [26]. Our study showed the maternal age as a risk factor as the chromosomal anomalies were found in patients born to mothers with age above 40 years and less than 20 years.

Consanguinity is another factor that determines genetic defects in the offspring. Bermudez [27] reports a study on 48 patients with ARM in which 56.3% of the patients were born to consanguineous couples. Siting the order of birth of the babies 27% were the first babies and 73 % were 2nd or above. However, in our study, only 22% of the patients were born to consanguineous couples and 50% of the babies were first in the birth order.

CONCLUSION

The Krickenbeck classification improves the understanding, management, and communication about anorectal malformations, thereby improving patient care and outcomes. According to the Krickenbeck classification Rectobulbar fistula and vestibular fistula were the most recurrently found ARMs. Karyotyping plays a vital role in the comprehensive evaluation of patients with anorectal malformations by identifying chromosomal abnormalities, guiding management decisions, providing prognostic information, and enabling genetic counseling. Individuals with Down syndrome have a higher risk of being born with anorectal malformations compared to the general population.

Conflict of Interest: Nil.

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