Original article

Cytogenetic analysis of children with congenital dysmorphism reported to tertiary care hospital in Lahore, Pakistan

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ABSTRACT

Background: The chromosomal imbalances are often seen in association with congenital dysmorphism. The identification of such chromosomal abnormalities is important, both as regards clinical management and for accurate genetic counselling. The current study sought to determine the association of chromosomal abnormalities to different groups of dysmorphisms.

Patients and Methods: This one-year descriptive cross-sectional study was conducted at The Children's Hospital and the Institute of Child Health, Lahore. Total 100 children between the age of 1 day to 12 years, reported with congenital dysmorphism were included in this study. Physical examination of the patients was carried out to note down their dysmorphic features. The blood samples of these children were taken to culture cells for chromosomal analysis by the G-banding method.

Results: Physical examination of the 92 patients revealed isolated dysmorphism in 14% and multiple dysmorphisms in 86% of the cases. Chromosomal abnormalities were found in 23.9% of the total patients. Among them, 7.7% with isolated and 26.5% cases with multiple malformations had chromosomal abnormalities.

Conclusion: This study concluded that both isolated and multiple congenital dysmorphisms are significantly associated with chromosomal abnormalities, therefore, chromosome analysis should be part of initial investigations of all the dysmorphic children.

Keywords:

Congenital dysmorphism; chromosomal abnormalities; dysmorphic children.

INTRODUCTION

The dysmorphism found at birth is known as congenital dysmorphism. It is found in approximately 3-4% of newborns and is a common cause of medical intervention, long-term illness and death.[1-4] In spite of the advancements in knowledge of the pathophysiology of congenital dysmorphism, the rate of the infant mortality due to major congenital malformations is about 22%.[5] Children with congenital dysmorphism may have internal or external malformations or developmental delay or combination of these. Diagnosis of such malformations is essential for the management, prognosis and calculating risk of recurrence.[6]

The cytogenetic analysis is an important aid in the diagnosis of a child with dysmorphic features.[7] Many etiological factors of congenital dysmorphism have been reported but chromosomal abnormalities play a significant role in their occurrence.[8,9] Although, the chromosomal abnormalities affect 7.5% of all conceptions, their frequency in the live births is only 0.7%.[8,9] It was reported that usually multiple malformations had chromosomal aetiology while the malformations of single organ showed multifactorial inheritance, implying the interaction of many genes with other factors.[10] Isolated dysmorphism is often missed or regarded as unimportant as regards chromosomal aberration as their underlying cause.

The present study was conducted to find out the significance of chromosomal analysis in children with isolated and multiple dysmorphisms.

Competing interest: The authors have declared no competing interests exist.
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PATIENTS AND METHODS

This one-year descriptive cross-sectional study was conducted at the Medical Genetics Department of the Lahore. Children's. Hospital and the Institute of Child Health, Lahore. Total 100 children between 1 day to 12 years of age and having some form of detectable congenital dysmorphism were included in this study. These patients were referred to the Cytogenetics Laboratory of Medical Genetics Department for chromosomal analysis from the Departments of Neonatology, General Medical units and from OPD of the Children's Hospital and the Institute of Child Health, Lahore. A detailed history from the parents of dysmorphic children was obtained and physical examination was carried out to note down their dysmorphism. The patients were divided into two groups. Those having involvement of single system were grouped under isolated congenital

dysmorphism group and the other that had malformations involving more than one system was grouped in multiple congenital dysmorphism group. The blood samples from the study subjects were collected in heparinized tubes. The cytogenetic analysis was performed on cultured peripheral blood lymphocytes stimulated with phytohemagglutinin, using standard techniques described by Rooney and Czepulkowski.[11] The karyotype was determined in all patients by G-banding. At least 10–15 metaphases were analyzed for each patient. Up to 30 metaphases were analyzed if there was suspicion of a mosaic pattern of a chromosomal defect. The best metaphases were photographed to determine their karyotypes. Those cases with culture failure during processing were excluded from the study. Data analysis was done by using SPSS. The significance of association of chromosomal abnormalities with both groups of congenital dysmorphism was carried out by applying the χ^2-test.

RESULTS

A total of 100 patients reported with congenital malformations were recruited for this study over a period of one year. Blood samples were taken from all the patients for cytogenetic analysis. However, eight cases were later excluded from the study due to culture failure. Amongst remaining 92 dysmorphic children, 52 (57%) were male and 40 (43%) were females having a mean age of 2.3 years (range = 1 day to 12 years). Age group distribution showed that 27 (29%) were less than 1 month old, 30 (33%) were 1 to 12 months old, 17 (18%) were 13 to 60 months (>1 to 5years) old while 18 (20%) were > 61 months (>5 years) old.

Consanguinity of the parents was present in 59 (64%) patients. The family history of the disease was recorded in 35 (38%) patients out of which 24 (69%) were born to consanguineous parents. Physical examination revealed presence of isolated congenital dysmorphism in 13 (14%) patients while multiple dysmorphisms were seen in 79 (86%) patients

Amongst cases of isolated congenital dysmorphism, craniofacial dysmorphism was observed in 11 (84.6%) patients while limbs dysmorphism was reported in 2 (15.4%) patients. Different combinations of dysmorphism were observed in patients with multiple congenital dysmorphisms. The craniofacial dysmorphism in combination with other types of dysmorphisms was the commonest present in the 78 (99%) patients. In 43 (54.4%) patients craniofacial malformations were associated with limbs malformations, 10 (12.7%) patients with limbs and CVS malformations, 6 (7.6%) patients with limbs and genitourinary malformations, 4 (5.1%) patients with limbs and chest malformations, 2 (2.5%) with limbs and CNS, 2 (2.5%) with limbs and abdomen malformations, and 1 (1.3%) patient with limbs, chest and abdominal malformations. Craniofacial malformations along with CVS malformations were present in 4 (5.1%) patients. Similarly, 4 (5.1%) patients had craniofacial and chest malformations while 2 (2.5%) patients had craniofacial and CNS malformations. Craniofacial and genitourinary malformations were recorded in 1 (1.3%) patient while 1 patient (1.3%) had only limbs and genitourinary malformations. Table 1 shows frequency of different types of malformation observed in children.

Cytogenetic analysis of 92 patients revealed presence of abnormal chromosomes in 22 (24%) patients. Among them, 1/13 patient (7.7%) having isolated congenital dysmorphism had detectable chromosomal abnormalities (p-value=0.004), while 21/79 (26.6%) of the cases of multiple congenital

Table 1. Frequency of various types of malformations observed in the patients

Type of Malformations	Frequency (%)
Isolated malformations (N=13)	
Craniofacial	11 (84.6)
Limbs	2 (15.4)
Multiple malformations (N=79)	
Craniofacial, limbs	43 (54.4)
Craniofacial, limbs, CNS	2 (2.5)
Craniofacial, limbs, CVS	10 (12.7)
Craniofacial, limbs, genitourinary	6 (7.6)
Craniofacial, limbs, chest	4 (5.1)
Craniofacial, limbs, abdomen	2 (2.5)
Craniofacial, limbs, chest, abdomen	1 (1.3)
Craniofacial, CNS	2 (2.5)
Craniofacial, chest	4 (5.1)
Craniofacial, CVS	4 (5.1)
Limbs, genitourinary	1 (1.3)

Table 2. Chromosomal abnormalities observed with isolated and multiple congenital dysmorphism in the dysmorphic children

Type of Malformations	Chromosomal Abnormalities	Diagnosis	Frequency (%)
Isolated dysmorphism – structural dysmorphism			
Craniofacial	46,XX,del(Xq)	Unknown	1 (4.5)
Multiple dysmorphisms – numerical abnormalities			
Craniofacial, limbs, CVS	47,XX,+21 or 47,XY,+21	Down syndrome	12 (54.5)
Craniofacial, limbs, genitourinary	46,XY/47,XY,+21	Down syndrome	1 (4.5)
Craniofacial, limbs, CVS	45,XO	Turner syndrome	1 (4.5)
Craniofacial, limbs	45,XO/46,XX	Turner syndrome	1 (4.5)
Craniofacial, limbs, CVS	47,XXY	Klinefelter syndrome	1 (4.5)
Craniofacial, limbs	47,XY+18	Edward syndrome	1 (4.5)
Multiple dysmorphisms – structural abnormalities			
Craniofacial, limbs	46,XY,t(q21;q21)	Down syndrome	1 (4.5)
Craniofacial, limbs, CVS	46,XY,del(7)(q32→q34)	Unknown	1 (4.5)
Craniofacial, limbs	46,XY,i(9q)	Unknown	1 (4.5)
Craniofacial, limbs	Partial trisomy of chromosome 8	Unknown	1 (4.5)

dysmorphisms had chromosomal abnormalities (p-value=0.0001). Table 2 shows different types of chromosomal abnormalities observed with isolated and multiple congenital dysmorphisms.

Among total 21 multiple dysmorphic participants, 17 (80.9%) had numerical chromosomal abnormalities. Among them, family history of disease was present in 5 (29.4%) patients and consanguinity was present in 10 (58.8%) patients. Trisomy 21 (Down syndrome) was recorded in 13 (76.5%) patients including regular trisomy (47,XX,+21 or 47,XY,+21) in 12 (92.3%) and mosaicism (46,XY/47,XY,+21) in 1 (7.7%) patient. Edward syndrome (47,XY,+18) was diagnosed in 1 (5.8%) patient while monosomy X was diagnosed in 2 (11.7%) patients. Two patients were diagnosed with Turner syndrome, 1 with classic karyotype (45, XO) and 1 with somatic mosaicism (45, XO/46, XX). Klinefelter syndrome (47, XXY) was diagnosed in 1 patient (5.8%).

Structural chromosomal abnormalities were observed in 5/21 (23.8%) patients. Consanguinity of the parents was present in 2 (40%) patients and 1 (20%) of them was having the family history of disease as well. Robertsonian translocation between the long arms of two chromosomes 21 (46,XY,t(q21;q21)) was seen in 1 (20%) patient diagnosed as a case of Down syndrome. Deletion on chromosome 7 was detected in 1 (20%) patient (46,XY,del(7)(q32→q34)). Similarly, deletion was detected in 1 (20%) patient on the long arm of X chromosome (46,XX,del(Xq)). Formation of isochromosome of the long arm of chromosome 9 [46, XX, i(9q)] was seen in another patient. In the remaining 1 case, the short arm of chromosome 2 had an insertion of a part of short arm of chromosome 8. On analysis of the parent's karyotype, it was seen that he inherited the defective chromosome 2 from father while patient had the normal pair of chromosome 8. This results in partial trisomy of 8p in the patient.

DISCUSSION

Several studies have reported different frequencies for various groups of congenital dysmorphism. A study carried out in Iran showed that the dysmorphisms involving the musculoskeletal system were the most frequent (35%) and the majority of these were isolated.[12] Similarly, another study done in Indian population by Swain and colleagues[13] reported that isolated dysmorphisms were present in 53% cases with CNS being the most commonly involved (39.5%). Moreover, multiple dysmorphisms were observed in 18.8% of cases. Another study revealed that multiple anomalies were present in 16.7% infants and the commonly affected system was CNS (48.8%).[14] Asindi and colleagues[15] reported that congenital malformations were present in 35.5% of the patients and alimentary canal was the most affected (56.8%). In the present study, multiple congenital dysmorphisms were commonly reported in 86% of the patients while 14% had isolated dysmorphism. In both groups of dysmorphism, craniofacial dysmorphism was the commonest with 85% children affected in isolated dysmorphism group and 98.7% in multiple congenital dysmorphism group. These results differ from previous studies, as described above. The differences may be because of the influence of geographical, racial or cultural differences. However, geographical evidence in favor of factors as a cause of congenital dysmorphism is lacking. The present study has shown a higher incidence of malformation in children of population with higher rate of consanguineous marriages. The frequency of consanguinity was found to be 65%. This frequency is in agreement with the study done by Muthukumaravel and colleagues[16] showing 73.3% frequency of consanguinity of parents in malformed children. Bhat and Babu[17] demonstrated a significant association with parental consanguinity and malformations (p-value<0.001). The chromosomal abnormalities were recorded in 22 out of 92 (23.9%) patients, which is close to the frequencies reported by Santos and colleagues[3] (26%), Verma and Dosik[18] (27.7%) and Alarrayed[19] (27%). However, Dinesh and colleagues[20], Mokhtar[9], Shah and co-workers[21] reported higher frequencies (38.7%, 41% and 39.6% respectively). Nevertheless, the frequency reported in this study is still higher than the frequencies observed in many other studies (16% approx.).[22-24] The heterogeneity in the frequencies of chromosomal abnormalities among these studies is probably due to variations in skill, facilities and techniques used for chromosomal analysis; cultural or ethnic reasons and need to be sought. In this study, the chromosomal abnormalities were detected in 8% cases of isolated congenital dysmorphism (p-value=0.004) and 26% of patients with multiple congenital dysmorphism (p-value=0.0001). The data is consistent with a study done in Belgium[25] which showed a similar frequency of chromosomal abnormalities in case of isolated malformations (9.3%) but less in case of multiple malformations (18.8%).

CONCLUSION

This study concludes that chromosomal defects are significantly associated with both isolated and multiple congenital dysmorphism. Therefore, it is recommend that for better management and counselling, chromosomal analysis should be done in all cases of congenital dysmorphism. In the remaining patients where no chromosomal abnormality can be detected, other possible causes of dysmorphism e.g. single gene defect, multifactorial or environmental factors need to be probed in order to find possible management strategies.

REFERENCES

1. Kalter H, Warkany J. Congenital malformations: etiologic factors and their role in prevention. N Engl J Med 1983;308(8):424-31.
2. Brent RL. Environmental causes of human congenital malformations: the pediatrician's role in dealing with these complex clinical problems caused by a multiplicity of environmental and genetic factors. Pediatrics. 2004;113(4 Suppl):957-68.

3. Santos C, Boy R, Santos J, Silva M, Pimentel M. Chromosomal investigations in patients with mental retardation and/or congenital malformations. Genet Mol Biol 2000;23(4):703-7.
4. Hudgins L, Cassidy SB. Congenital anomalies. In: Martin RJ, Fanaroff AA, Walsh MC, editors. Neonatal- Perinatal Medicine. 8th ed. Philadelphia: Mosby-Elsevier; 2006. p.561-81.
5. Lee K-s, Khoshnood B, Chen L, Wall SN, Cromie WJ, Mittendorf RL. Infant mortality from congenital malformations in the United States, 1970–1997. Obstetrics & Gynecology. 2001;98(4):620-7.
6. Kingston HM. ABC of clinical genetics. Dysmorphology and teratogenesis. BMJ 1989;298(6682):1235-1239.
7. Hunter AG. Medical genetics: 2. The diagnostic approach to the child with dysmorphic signs. CMAJ 2002;167(4):367-72.
8. Goud MT, Al-Harassi SM, Al-Khalili SA, Al-Salmani KK, Al-Busaidy SM, Rajab A. Incidence of chromosome abnormalities in the Sultanate of Oman. Saudi Med J 2005;26(12):1951-7.
9. Mokhtar MM. Chromosomal aberrations in children with suspected genetic disorders. East Mediterr Health J 1997; 3(1):114-122.
10. Stevenson RE, Hall JG, Goodman RM. Human malformations and related anomalies. New York: Oxford University Press; 1993.
11. DE Rooney, Czepulkowski B. Human cytogenetics: A practical approach. 2nd ed. New York: Oxford University Press; 1992. p.39-55.
12. Ali A, Zahad S, Masoumeh A, Azar A. Congenital malformations among live births at Arvand Hospital, Ahwaz, Iran-A prospective study. Pak J Med Sci 2008;24(1):33.
13. Swain S, Agrawal A, Bhatia B. Congenital malformations at birth. Indian Pediat 1994;31(10):1187-91.
14. Al-Jama F. Congenital malformations in newborns in a teaching hospital in eastern Saudi Arabia. J Obstet Gynaecol. 2001;21(6):595-8.
15. Asindi AA, Al Hifzi I, Bassuni W. Major congenital malformations among Saudi infants admitted to Asir Central Hospital. Ann Saudi Med 1997;17:250-3.
16. Muthukumaravel N, Ramachandra RK, Vishnu BB. Cytogenetic studies in infants with congenital malformations. Curr Pediatr Res 2005;9:11-14.
17. Bhat BV, Babu L. Congenital malformations at birth—a prospective study from south India. Indian J Pediatr 1998;65(6):873-81.
18. Verma RS, Dosik H. Incidence of major chromosomal abnormalities in a referred population for suspected chromosomal aberrations: a report of 357 cases. Clin Genet 1980;17(5):305-8.
19. Alarrayed SS. Chromosomal abnormality in Bahrain (6 years study). Am J Hum Genet 1991; 49(4):255(1399).
20. Dinesh RD, Pavithran K, Henry P, Elizabeth K, Sindhu P, Vijayakumar T. Correlation of age and birth order of parents with chromosomal anomalies in children. Russ J Genet 2003;39(6):695-9.
21. Shah V, Mutiny DK, Murthy SK. Cytogenetic studies in a population suspected to have chromosomal abnormalities. Indian J Pediatr 1990;57(2):235-43.
22. Kenue R, Raj AG, Harris P, El-Bualy M. Cytogenetic analysis of children suspected of chromosomal abnormalities. J Trop Pediatr 1995;41(2):77-80.
23. Kim HK, Koo NM, Hwang JS, Kim SH. Review of chromosomal analysis performed in single hospital. Korean J Paediatr 2004;4:1157-60
24. Navsaria D, Mathews T, Conte RA, Verma RS. Chromosomal anomalies in 1,000 children referred with suspected genetic disorders. Hum Hered 1993;43(3):137-40.
25. Staebler M, Donner C, Van Regemorter N, Duprez L, De Maertelaer V, Devreker F, et al. Should determination of the karyotype be systematic for all malformations detected by obstetrical ultrasound? Prenat Diagn 2005;25(7):567-73.