# CHROMOSOMAL ABNORMALITY IN INDIVIDUALS WITH CLEFT LIP OR CLEFT PALATE

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***ABSTRACT:***

*Cleft lip or palate (CL/P) is one of most common congenital anomalies. The worldwide incidence of CL/P is 1 in 700 and in India it is 1 in 500 live births. Of the various etiological factors, chromosomal aberrations are reported as one of the major causes. Hence, the main objective of this study was to screen for the presence of chromosomal aberrations in individuals with cleft lip or palate or both of Indian origin. The blood samples were obtained from 10 patients visited the departments of Plastic and Reconstructive surgery and Human Genetics, Sri Ramachandra University, with informed consent. The chromosomes were analyzed from the cultured lymphocytes after GTG banding. The abnormality identified with GTG banding was confirmed using FISH. Of the ten cases screened for chromosome abnormality, nine showed normal karyotype and one with trisomy 18. The trisomy was confirmed with FISH using locus specific probe for chromosome # 18. Since cleft lip and palate belong to the multifactorial group, the influence of other factors in the causation of cleft lip and palate cannot be ruled out in the cases with normal karyotype.*

***Key words:*** Cleft plate, Cleft lip, GTG banding, FISH.

## INTRODUCTION

Congenital malformations are defect and /or cognitive delays present at the time of birth. The defects may be an isolated or syndromic. Approximately 2% of live births have major congenital malformations. The etiologies for such malformations include single gene defects (20%), chromosomal aberrations (10%), teratogens (10%), environmental factors (30%) and other unknown causes (30%) (1). Cleft lip and palate are one of the most common congenital malformations. While, the incidence of CL/P worldwide is 1 in 700 live births and it is nearly 1 in 500 in India (2). Thus, the incidence of cleft lip and palate varies according to geographical location, ethnicity and socio-economic status (3).

Cleft lip can occur either as unilateral (left or right side) or bilateral anomaly. Furthermore, CLP can be isolated, non-syndromic (70%) or it can be syndromic where more additional anomalies are involved along with CL/P. The majority of the CL/Ps is non-syndromic (70%) and the remaining are syndromic cases. Orofacial clefts represent a complex phenotype which can be caused by many etiological factors. In a large series of cases, it has been found that they are caused by single mutant genes, chromosomal aberrations, specific environmental agents or interaction of many genetic and environmental factors, the multifactorial group (4). The high familial aggregation rates, recurrence risks and elevated concordance rates in monozygotic twins provide evidence for a strong genetic component in CL/P (5). Environmental causes includes Teratogens (Maternal smoking), infections, nutrients (folic acid supplement) and cholesterol metabolism (has role in human facial embryogenesis) (6).

Fogh-Anderson (1942) provided the first population-based evidence that CLP (cleft of the lip or palate) has a strong genetic component (7). Studies have been reported the association between chromosomal anomaly and clefts of the palate in animals and humans. Ingalls (1963) induced cleft palate in mice by administrating 6-amino nicotinamide to pregnant females and found polyploidy and fragmentation of chromosomes in fetuses affected with isolated cleft palate. Gropp et al (1964) reported a patient with cleft palate showed nearly triploid chromosomes with modal number of 72 chromosomes in cells cultivated from palatal mucosa. On contrary, negative association of chromosomal aberration and CL/P is also reported (8&9). In the view of different studies, in the present we have reported the results of chromosomal studies carried out from 10 patients with CL/P of south Indian origin. The chromosomes were analyzed using GTG banding and FISH.

## AIM

The main aim of the study was to screen for the presence of chromosomal abnormality in individuals with cleft lip or cleft palate or both by Giemsa staining.

## MATERIALS AND METHODS:

The study group involved 10 individuals with cleft lip or palate or both attended the Departments of Plastic and Reconstructive surgery and Human Genetics, Sri Ramachandra University. A pedigree and medical history was charted out from the data provided by the patient and/or guardian. The information and the blood samples were collected from the informed consent of the patient or/ and patient's guardian. About 5ml of peripheral blood was drawn in heparinized vacutainers and used for chromosomal study as explained below.

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***Chromosome preparation and GTG- Banding***

About 1ml of blood was added to 8 ml of RPMI medium, 2ml of fetal bovine serum and 500 microlitre of Phytohaemagglutinin and incubated at 37°C with 5% $CO_2$ for 72 hours. At 66.5 hour, Ethidium bromide (1mg/ml) was added followed by the addition of Colchicine (0.1mg/ml) at 67th hour and incubated for 1.5 hour. The cells were then harvested by hypotonic treatment (20 minutes with 0.45%KCL at 37°C), washed thrice with Carnoy's fixative (methanol and acetic acid 3:1) and casted on clean prechilled slides. Multiple slides were casted for each sample and used for chromosomal aberration analysis and Fluorescence in –situ hybridization. The slides were exposed to Trypsin (8mg/50 ml of Nacl) for 20 – 30 seconds and then stained with 10% Giemsa, air dried and mounted with coverslip using DPX for the analysis of chromosomal aberrations. For each sample 25 metaphases were analyzed and interpreted (10).

***Fluorescence in-situ hybridization:***

The slides with metaphase chromosomes prepared as mentioned above was dehydrated in 70%, 80% and100% ethanol for 2min each, at room temperature and air-dried. The locus specific probe was mixed with hybridization buffer and deionised distilled water, and applied to the slides. The metaphase chromosomes and the probes were co-denatured using Hybrite at 73°C for 3 minutes. The slides were sealed with coverslip using rubber cement and hybridization was carried out for 24 hours at 37°C. After 24 hours of hybridization, the coverslip was removed and the slides were rinsed in formamide wash solution (0.4X SSC/0.3%NP-40) at 45°C and the slides were air-dried. After air drying the slides were counterstained with DAPI (7.5µl/slide) and covered with coverslip and slides were stored in dark prior to signal enumeration and observed under fluorescent microscope for appropriate signals (11).

**RESULTS:**

Table-1 gives the details of patient age, sex, consanguinity and type of CLP. The age group varies between 2 days to 19 years. Among the cases screened, 5 patients are with cleft palate, 2 patients with unilateral cleft lip, 2 patients with bilateral cleft lip and palate and 1 patient with complete cleft lip and palate

Of the 10 patients, 3 were born to the parents of first degree consanguinity marriage and remaining seven of them were non-related. Only one patient's mother had the medical history of intake of Dolopar (Acetaminophen) during pregnancy. The cases 9 and 10 are examples of cleft related syndromes and the rest are examples of non-syndromic cleft lip and/or palate.

Twenty five G-banded metaphases at 450-550 band resolution were analyzed for each patient. The karyotypes of the patients were given in table-1. The result showed 9 cases with normal karyotype and one with trisomy-18.

**Table 1 : Profile of study subjects**

| Code number | Age/sex | Consanguinity | Cleft type | Karyotype |
|---|---|---|---|---|
| Case 1 | 14 yrs/ M | 1st Degree | Incomplete cleft of soft palate | 46,XY |
| Case 2 | 8 yrs/F | N C | Complete cleft of hard and soft palate | 46,XX |
| Case 3 | 13yrs/ M | N C | Complete cleft of hard & soft palate | 46,XY |
| Case 4 | 8yrs/M | N C | Unilateral cleft lip | 46,XY |
| Case 5 | 3 yrs/F | N C | Unilateral cleft lip | 46,XX |
| Case 6 | 12yrs/M | N C | Bilateral complete cleft lip & palate | 46,XY |
| Case 7 * | 17yrs/F | N C | Bilateral cleft lip & palate | 46,XX |
| Case 8 | 19yrs/F | 1st degree | Complete cleft of posterior & soft palate | 46,XX |
| Case 9 | 11mon/M | 1st degree | Cleft palate | 46,XY |
| Case 10 | 2days/F | N C | Complete cleft lip & palate | 47,XX,+18 |

* Medical history of drug intake during pregnancy

NC – Non consanguineous

Karyotype of the patient with trisomy 18 was further confirmed by increasing the analysis of G-banded metaphases to 100 and by FISH (Figure 1a & 1b).

**Case 10**
**47, XX, + 18**

Fig. 1 a

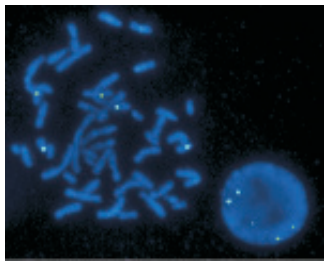
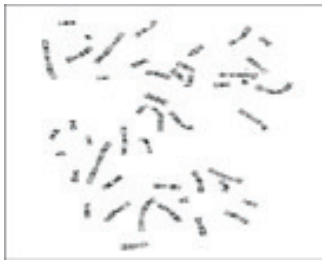


Fig. 1 b



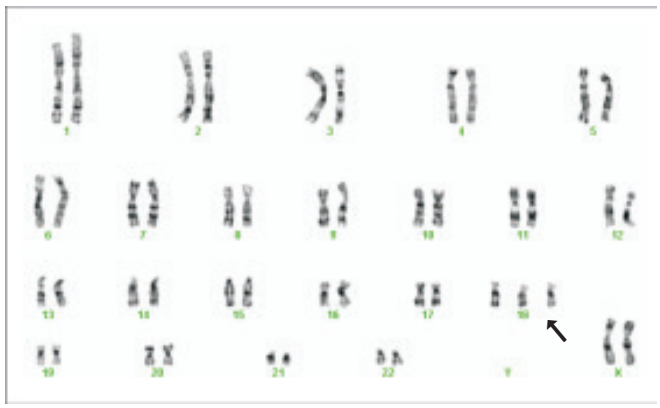


**Fig. 1a** : Fish with Locus specific probe for chromosome # 18 showing trisomy of Chromosome 18

**Fig. 1b** : GTG banded metaphase showing Trisomy 18

## DISCUSSION

It has been estimated that 6% of all congenital malformations are due to visible cytogenetic abnormalities (12). Of which approximately 5% of congenital defects with cleft lip and/ or palate have been reported an association with structural and numerical chromosomal abnormalities (3). Chromosomal aberrations either numerical or structural can be identified by the GTG banding technique with a band resolution of 400-450. This is the widely used cytogenetic method to screen the genetic association for different malformations. In an attempt to screen for chromosomal abnormalities in individuals with CL/P, consequently gains an insight to the possible relation between the two, of the ten cases screened. Nine out of ten cases showed normal karyotype and one with trisomy 18. Earlier, Subrt et al reported ten negative results and one trisomy 21 karyotype out of eleven cases studied.

In the present study, of the ten cases screened, one of them (case – 10) showed trisomy of chromosome 18. Aneuploidies occur due to non-disjunction of chromosomes during meiosis, resulting in an extra chromosome than the usual two copies. Therefore, the presence of an extra copy of genes on these chromosomes, results in multiple malformations leading to a syndrome. Trisomy 18 has been associated with the presence of cleft lip and/or palate (13&14). Clefts of lip or palate or both have also been observed in individuals with ring chromosome 18 (1) and chromosome 18 involved in a complex rearrangement (15). This shows that chromosome 18 might be harbouring a gene (or genes) that have a direct role in lip and/or palate formation, or atleast acts as a modifier during embryogenesis.

Case 9 presented with the clinical features like micrognathia, cleft palate, and glossoptossis which are characteristic of Pierre Robin Syndrome (also referred to as Pierre Robin Sequence). He was the child of consanguineously married parents. Pierre Robin Syndrome occurs sporadically, but it may be familial, in which the mode of inheritance is autosomal dominant (16). However, GTG banding technique at 450-550 band resolution may not be sensitive enough to detect complex alterations, submicroscopic deletions or single gene changes, which may be causative reason(s) of CL/P. This could be one of the reasons for failing to identify subtle chromosomal alterations often associated with CL/P.

Individuals, whose karyotypes showed no numerical or visible structural abnormalities, two (cases – 1 and 8) were children of consanguineously married parents. Significant association has been found between clefting and consanguinity (17). CL/P can be due to either an insult as gross as a visible chromosomal alteration or changes in genes as subtle as substitution, deletion, etc which cannot be detected by conventional cytogenetic techniques. Micro deletions or isodisomy may also contribute to clefts as suggested by studies. Hence using techniques like Comparative genomic hybridization (CGH) or mFISH can detect such cross chromosomal abnormalities which were missed by conventional karyotyping in the above individuals. Moreover, it is shown that number of genes has been associated with the regulation and craniofacial morphogenesis (6). Perturbations in the function of any of these genes in the form of mutations can result in haploinsufficiency leading to a cleft lip, or palate, or both depending on the affected gene and its role during embryogenesis (18). Thus the role of genes in regulating the morphogenesis could not be identified in the present study.

Of the other four cases, there were two pairs of siblings (cases – 2, 3 and 4, 5). Neither of the pairs of the siblings had any other family member affected. The manifestation of the disease in these individuals could be due to a possible de novo germ line mutation of any of the related genes in either of the parents or they could have been subjected to some environmental factors during their embryogenesis (life style habits and health of the mother during pregnancy). Also, the contribution of syndromic genes in these non-syndromic cases cannot be ruled out.

The negative results obtained for the cases screened (cases 1-9) could mainly be attributed to the fact that CL/P is a complex anomaly with a multifactorial inheritance. Though there are many genes involved in the formation of the lip and palate during embryogenesis, the intrauterine environmental factors and other environmental factors like maternal smoking, consumption of drugs (teratogens), and nutrition also have an influence on the developing fetus, which should also be considered.

## CONCLUSION:

To identify presence of subtle chromosomal alterations in complex disorders like the cleft lip and palate if any, karyotyping has to be combined with new techniques. This would increase the sensitivity of the diagnosis and hence rule out the genetic contribution; as the recurrence risk of cleft lip and palate increases in siblings of affected individuals with chromosomal/genetic abnormalities. Once the genetic contribution is ruled out, the other environmental factors (maternal smoking, maternal nutritional status, uptake of teratogenic drugs) which could have been plausible causes can be tried and identified so that these factors can be modified/avoided in subsequent pregnancies.

In spite of limited efficiency of karyotyping in detecting subtle chromosomal aberrations, it still serves as the basis for identifying gross chromosomal aberrations and hence aids in ruling out chromosomal aberrations as the possible cause for malformation like that of CL/P. Consequently it helps directing individuals with such complex disorders in the right path of diagnosis, by leading them to look at the newer aspects of other causes.

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**REFERENCES:**

1. Gustavson KH, Hagberg B, Finley SC, Finley WH. An apparently identical extra autosome in two severely retarded sisters with multiple malformations. Cytogenetics 1962;Vol.1, :32-41.
2. Uppala R, Gaines M, Beiraghi S, Hutchings D, Golla J, Husain SA. Genomewide scan for nonsyndromic cleft lip and palate in multigenerational Indian families reveals significant evidence of linkage at 13q33.1-34. Am J Hum Genet 2006 Sep; 79:580-585.
3. Cobourne MT. The complex genetics of cleft lip and palate. Eur J Orth 2004; 26:7-16.
4. Little J, Cardy A, Munger RG. Tobacco smoking and oral clefts: a meta-analysis. Bull World Health Organ 2004; 82:213–218.
5. Stanier P, Moore GE. Genetics of cleft lip and palate: syndromic genes contribute to the incidence of non-syndromic clefts. Hum Mol Gen 2004 Jan 13; 13(1): 73-81.
6. Iris ALM, Keers CV, Kluijtmans LAJ, Ocke MC, Zielhuis GA, Brouwer SMG, et al. Does the interaction between maternal folate intake and the methylenetetra hydrofolate reductase polymorphisms affect the risk of cleft lip with or without cleft palate Am J Epidem 2003;157
7. Beiraghi S, Zhou M, Talmadge CB. Identification and characterization of a novel gene disrupted by a pericentric inversion inv (4) (p13.1; q21.1) in a family with cleft lip. Gene 2003; 309(1):11-21.
8. Makoni S. Chromosomal studies in normal subjects and in 300 cases of congenital disorders. Part II. Cytologia 1964; 29:125-50.
9. Subrt I, Cervenka J, Krecek M. Cytogenetic study of cleft lip and palate. Meeting of the Czechoslovak Surgical Congress, Bratislava; 1965.
10. Shaffer L G, and Tommerup N, Karger S, Basel. ISCN, 2005. An international System for Human Cytogenetic Nomenclature. 2005. pp-1-127.
11. Venkatachalam P, Jayanth V R, Solomon F D Paul, Vettriselvi V. Protective effect of 2-deoxy-D-glucose on chemotherapeutic drugs induced damages on peripheral blood lymphocytes exposed in- vitro. Int J Hum Genet, 2006; 6: 133-138.
12. Patau K, Smith DW, Inhorn SL. Two new cases of $D_1$ – trisomy in man. Hereditas 1961; 47:239.
13. Lucas M, Kemp NH, Ellis JR, Marshall R. A small autosomal ring chromosome in a female infant with congenital malformations. Ann Hum Genet 1963; 27:189-195.
14. Van Wijck JAM, Stolte LAM, Van Kessel HIAM, Tijdink. A trisomy child of a hyperthyroid mother. Lancet 1961; 1:887-88.
15. Brewer C, Holloway S, Zawalnyski P, Schnizel A, FitzPatrik D. A chromosomal duplication map of human malformations: regions of suspected haplo- and triplolethality- and tolerance of segmental aneuploidy – in humans. Am J Hum Genet 1999; 64:1702-8.
16. Stoll C, Alembik Y, Dott B, Roth MP. Epidemiological and genetic study in 207 cases of oral clefts in Alsace, north-eastern France. J Med Genet 1991; 28:325-29.
17. Tachdjian G, Aboura A, Lapierre JM, Viguie F. Cytogenetic analysis from DNA by comparative genomic hybridization. Ann Genet 2000; 43:147-54.
18. Sliuzas V, Cimbalistiene L, Kucinskas V. Patient with syndromic cleft lip-palate, mosaic karyotypes and cytogenetically abnormal brother. Acta Medica Lituanica 2006; 13: 97-104.
19. Brewer C, Holloway S, Zawalnyski P, Schnizel A, FitzPatrik D. A chromosomal duplication map of human malformations. Am J Hum Genet 1998;62:1153-9.
20. Gropp A, Jussen A, Odunjo F. Near-triploid chromosome constitution in epithelial-cell cultures of palatal mucosa from a case of cleft palate. Lancet 1964; 1:1167.
21. Alkuraya FS, Saadi I, Lund JL, Turbe A, Morton CC, Maas RL. SUMO 1 haploinsufficiency leads to cleft lip and palate. Science 2006; 313:1751.
22. Stromme P, van der Hagen CB, Haakonsen M, Risberg K, Hennekam R. Follow-up of a girl with cleft lip and palate and multiple malformations: trisomy 20 mosaicism. Scand J Plast Reconstr Surg Hand Surg 2005; 39(3):178-9.
23. Ounap K, Ilus T, Laidre P, Uibo O, Tammur P, Bartsch O. A new case of duplication supports either a locus for orofacial clefting between markers D2S1897 and D2S2023 or a locus for cleft palate only on chromosome 2q13-q21. Am J Med Genet 2005 Sep 1;137(3):323-7.
24. Online Mendelian Inheritance in Man: http://www.ncbi.nim.gov/omim/OFC1
25. Online Mendelian Inheritance in Man: http://www.ncbi.nim.gov/omim.
26. Verma RS, ArvindBabu, McGraw-Hill. Human chromosomes- Principles and Techniques. Second edition. Inc, 1995.