Original Article - Analytical Study

Evaluation of 22q11.2 deletion in Cleft Palate patients

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ABSTRACT

Background: Cleft palate is the commonest multifactorial epigenetic disorder with a prevalence of 0.43–2.45 per 1000. The objectives of this study were to evaluate the clinical features and identify the 22q11.2 deletion in patients with cleft palate in Sri Lanka. **Materials and Methods:** Cleft patients attending a Teaching Hospital in Sri Lanka were recruited for this study. The relevant data were obtained from review of case notes, interviews, and examination of patients according to a standard evaluation sheet. Quantitative multiplex polymerase chain reaction (PCR) was performed to identify the 22q11.2 deletion. A gel documentation system (Bio-Doc) was used to quantify the PCR product following electrophoresis on 0.8% agarose gel. **Results and Conclusion:** There were 162 cleft palate patients of whom 59% were females. A total of 92 cleft palate subjects (56.2%) had other associated clinical features. Dysmorphic features (25.27%) and developmental delays (25.27%) were the commonest medical problems encountered. The cleft was limited to the soft palate in 125 patients, while in 25 patients it involved both the hard and the soft palate. There were seven subjects with bifid uvula and five subjects with submucous cleft palate. None of the patients had 22q11.2 deletion in this study population. A multicentered large population-based study is needed to confirm the results of this study and to develop guidelines on the appropriate use of 22q11.2 deletion testing, which are valid for cleft palate patients in Sri Lanka.

Keywords: 22q11 deletion syndrome, cleft palate, congenital anomalies, polymerase chain reaction, Sri Lanka

INTRODUCTION

Cleft palate is a congenital fissure in the roof of the mouth that results from incomplete fusion of the palate during embryonic development.[1] It is the most common congenital malformations of the head and neck region. It is often associated with cleft lip and various other congenital anomalies.[2] It contributes substantially to long-term disability in children, as well as tremendous emotional and financial stress for the affected families and individuals. The treatment is a long-term process that starts soon after the birth and continues well into the end of the second decade of life with multiple surgeries and long-term speech, orthodontics, audiological, medical, and dental care.

Development of the palate occurs between the 6th and 11th weeks of intrauterine life. Abnormalities of any of the critical events of development due to environmental, local, or genetic predisposition result in failure of the fusion of palatine shelves leading to clefts of the palate.[1,3]

Prevalence of cleft lip and palate

The prevalence of the cleft palate with or without cleft lip varies according to various factors. The overall incidence of cleft palate with or without cleft lip is 1 in 1000 live births.[4] Generally, the incidence of isolated cleft palate (without cleft lip) is 1 in 2000 live births. Submucous cleft palate is more common, with an incidence of 1 in 1200–2000 live births. The bifid uvula often occurs in isolation, without clefting of the palatal muscles.[5]

There are variations in the prevalence rates of cleft lip and palate in different regions. Low birth prevalence of clefts (0.24 per 1000 live births) was found in Zambia.[6] The prevalence rates of cleft

palate were reported to be 0.43 and 0.48 per 1000 live births in Australia and California, respectively.[7,8] The incidence of cleft lip and palate in Sri Lanka is 0.83 per 1000 live births, and the incidence of isolated cleft palate is 0.19 per 1000 live births. A positive family history has been found in 9.1% of cleft palate subjects in Sri Lanka.[9] The incidence of cleft lip and palate has been doubled during the last 50 years and tripled during the last 100 years.[10] A 30-year follow-up study showed a clear trend of rapid increase in cleft lip and palate in Finland.[11]

Etiology of cleft lip and palate

The etiology of cleft lip and palate is believed to be multifactorial. Several genetic and environmental factors interact with the process of morphogenesis of the primary and secondary palates.[9] Isolated cleft lip and palate unaccompanied by any other malformation is an autosomal dominant inherited disorder, and the genes were found to be located on the short arm of chromosome 6. Other pedigrees show autosomal recessive and X-linked recessive patterns.[12]

Trisomy 13, trisomy 18, velocardiofacial (VCF) syndrome, Pierre–Robin syndrome, fetal valproate syndrome, and oto-palato-digital syndrome are few of the syndromes that are associated with cleft palate.[2] There are over 400 syndromes which include cleft lip and/or cleft palate as a component and are listed in the London Dysmorphology Database.[2]

22q11 deletion syndrome

The chromosome 22q11 deletion syndrome (Mendelian inheritance in man database number 188400) is a relatively common genetic disorder characterized by congenital cardiac defects, cleft palate, velopharyngeal insufficiency, distinct facial features, immunological problems, learning disabilities, and psychological disorders.[5,13,14] This syndrome is caused by deletion of chromosomal material from the long arm of chromosome 22 (22q), which leads to a wide but variable spectrum of effects.

The term velocardiofacial syndrome was used for the milder end of this deletion syndrome. These patients usually manifest palatal anomalies, distinct facial features, and learning disabilities.[15] This disorder appears to occur as a result of failure or abnormalities in the formation of the 3rd and 4th branchial arch structures from which the affected organs and structures are derived.

22q11 deletion syndrome is one of the common syndromes associated with cleft palate. The prevalence of this syndrome has been estimated to be between 1 in 3800 and 1 in 6500 live births.[13,16] Among infants born with conotruncal heart defects, 5% have been found to have a deletion of chromosome 22q11.2.[16] Approximately 5–8% infants with cleft palate had a 22q11.2 deletion.[15] The prevalence of this deletion syndrome in Sri Lanka is not known.

The 22q11.2 region is a hotspot for rearrangements due to deletions, duplications, and translocations. These rearrangements result in altered gene dosage.[17-21] The most commonly deleted region of chromosome 22q11.2 involves the loss of a 3 Mb region in around 85% of cases, but a smaller nested deletion of 1.5 Mb is also described in a further 10% of cases.[14] The characteristic disease phenotype is caused by a haploinsufficiency of a series of 24–30 genes within the 22q11.2 region.[14]

This deletion occurs in about 94% of cases as a *de novo* event without preceding family history of a similar deletion. In about 6% of cases, the deletion is inherited from a parent.[14]

Diagnosis of 22q11 deletion syndrome is mainly based on the clinical evaluation and confirmed by laboratory investigations. Early detection of 22q11 deletion is far more important as potential complications related to this syndrome can be identified early for management of the condition prior to the cleft palate repair.[22]

The main objective of this study was to evaluate the clinical features and identify 22q11.2 deletion among patients with cleft palate in Sri Lanka.

MATERIALS AND METHODS

Patients with isolated cleft palate (without cleft lip) were selected for the study. Patients were identified among those who were currently under the review in the Regional Cleft Centre & Maxillofacial Department, Teaching Hospital, Karapitiya, Galle, Sri Lanka. All patients with isolated cleft palate registered in the clinic from 1 January 2001 to 31 December 2009 were included in the study. A total of 162 cleft palate patients participated in this study. Before enrolling in the study, the entire procedure of the research was briefly explained to the patients and in the case of children, to the parents or guardian. Steps had been taken to maintain the confidentiality of data. Before the evaluation of the patients, a written consent was obtained from all the patients and in case of the children, from parents or guardian.

The patients who consented to participate in the study were interviewed individually in detail by the researcher and data were recorded in an internationally accepted standard structured questionnaire. Complete evaluation of the patient was carried out including relevant history and full clinical examination. All the clinical notes and diagnosis cards were reviewed. Where necessary, patients were referred to special investigation units for procedures such as ultrasound scan, echocardiogram, computed tomography (CT) scan, magnetic resonance imaging (MRI) scan, hearing and visual investigations, etc. Feeding in infants and speech in older children and adults were evaluated by designated speech pathologist.

All the consented patients with cleft palate were included in the assessment of 22q11.2 deletion. One to three milliliters of venous blood was obtained from each patient for the molecular genetic analysis.

Ethical clearance was granted for the study by the Faculty of Graduate Studies, University of Keleniya, Sri Lanka.

Quantitative multiplex PCR

DNA was extracted by using commercially available human genomic DNA extraction kit (QIAamp DNA Mini Kit; Qiagen, Germany). Two sets of 300 µl of whole blood from each patient were used to extract DNA. All the extracted DNA samples were quantified by using UV spectrophotometer [Thermo Spectronic-Genesys (TM) 10].

Ten sets of forward and reverse primers were designed [Table 1] for the multiplex polymerase chain reaction (PCR) test for 22q11.2 deletion. Eight sets of primers were designed to the established sequence-tagged sites (STS) spanning between proximal and distal break points of the typically deleted region (TDR) of the 22q11.2 region, and two other set of primers were designed at the region of cystic fibrosis gene. Primers for the cystic fibrosis gene were used as an internal control outside the deleted region. All the designed primes were analyzed using Basic Local Alignment Search Tool (BLAST) for nonspecific alignments.

In order to detect the 22q11.2 deletion in patients, dosage analysis of markers within 22q11.2 region was carried out using PCR as described by Uddin *et al.* in 2006.[23] Eight sets of primers representing the established STS markers spanning the 3 Mb TDR were used for this purpose. PCR was carried out in a volume of 25 µl using a thermal DNA cycler (Eppendorf, Germany). Human genomic DNA (100 ng) from patients (P) and from a normal subject (N) were amplified using specific primer sets representing established STS markers spanning the 3 Mb TDR. For each PCR, an internal control of cystic fibrosis gene (SHGC35613) was also included. The annealing temperature for each primer set and the PCR conditions were optimized as described by Rolfs *et al.*[24] Quantification of PCR products was carried out in the log phase (30 cycles of PCR) after electrophoresis using a gel documentation system (Bio-Doc). All dosage estimations were carried out using three independent PCR reactions. A ratio of 1N:1P indicated that there was no deletion, while a ratio of 2N:1P indicated a deletion.

Table 1: List of primers used for the deletion testing

Seq. name	Given name	Sequence	Length (bp)	T_m
W13071fp	W1fp	GTAATTTTCTCCTACATTCTTAGG	25	56
W13071rp	W1rp	ATTATTTGCTCAACATTTAAAGAC	25	56
G18185fp	G1fp	TTCTCAACTCCCCCTGTCC	19	60
G18185rp	G1rp	CGATAGCAGTGAGGTGCAAA	20	60
D22S609fp	D1fp	ATCCCAAAGTACTTACAAAGCA	22	56
D22S609rp	D1rp	TGGGAGAGCTTGGAGTTTAA	20	58
D22S944fp	D2fp	CATGTGAAAGATGCTACTTTCC	21	55
D22S944rp	D2rp	ATCCCATGCTCCTCCCCAT	19	64
D22S931fp	D3fp	GTGAGATGGACCGGAACTTTG	21	62
D22S931rp	D3rp	CTACCAGGGCAATCCTGAGC	20	62
D22S264fp	D4fp	ATTAACTCATAAAGGAGCCC	20	53
D22S264rp	D4rp	CACCCCACCAGAGGTATTCC	20	62
SHGC14531fp	S1fp	TCCTGGATCTTACTAGTTTGCGG	23	62
SHGC14531rp	S1rp	TGATTGGAGATGAGTAAGCCACA	23	62
D22S936fp	D5fp	CAATCTTGGCAGCCAGTTTAG	21	60
D22S936rp	D5rp	CAGCATCTTCCTGGTGGCC	19	64
D22S636fp	D6fp	AACCTTCTGATGGCTCCTCT	20	58
D22S636rp	D6rp	CATGGAGCTGACACTGAGTG	20	58
SHGC35613fp	S2fp	TAAACCTCCCTGAAGAATCTTCC	23	60
SHGC35613rp	S2rp	AGACCAGAGCAGGGACAGAA	20	60

RESULTS

Gender, age and geographic distribution

There were 323 patients with cleft palate without cleft lip, who attended the Regional Cleft Centre & Maxillo-Facial department, Teaching Hospital Karapitiya over the period starting from 1 January 2001 to 31 December 2009. There were 187 females (57.9%) and 136 males (42.1%). By responding to the request to attend to the routine clinic review, 162 patients attended the study (50.14%). There were 95 (58.64%) females and 67 (41.36%) males.

The age range was from 2 weeks to 49 years. There were 24 patients with less than or equal to 1 year of age. Most of the patients were small children less than 5 years of age (51.23%).

Most of the subjects were from the Southern Province (90.12%) and majority of them were residing in the Galle district [Table 2].

Table 2: Geographic distribution

District	Number of patients	Percentage
Galle	84	51.85
Matara	39	24.07
Hambantota	23	14.20
Kaluthara	07	04.32
Colombo	05	03.08
Rathnapura	04	02.48

Type of cleft palate

There were 125 (77.16%) subjects with cleft soft palate. Twenty-five (15.43%) had cleft palate involving hard palate. Bifid uvula was the next prevailing condition involving 7 (4.32%) subjects. Five (3.09%) subjects with submucous cleft palate were also found among these patients.

Associated clinical conditions

Prevalence of other clinical conditions of the study population was evaluated. Ninety-two (56.79%) subjects had associated other clinical abnormalities. Out of these, 58 (63.04%) were males and 34 (36.96%) were females [Table 3].

Table 3: Gender distribution of other congenital abnormalities

Study population	Male (%)	Female (%)
Study sample	41.36	58.64
Subjects with other associated anomalies	63.34	36.96

Distribution of other congenital anomalies

Developmental delay and dysmorphic features were the commonest presentations occurring in 23 (14.2%) subjects each. The second most prevailing condition was cardiac malformation found in 15 (9.26%) subjects. Speech delay in 12 (7.07%), hearing and central nervous system abnormalities in 5 (3.09%) each, and epilepsy in 4 (2.47%) subjects were also noted. Genital, gastrointestinal, and renal anomalies were found in 2 (1.23%) subjects each. Visual abnormalities were seen in 1 (0.062%) subject [Figure 1].

Cardiac anomalies

Out of 15 subjects with congenital heart defects, 8 (53.33%) subjects with atrial septal defects (ASDs), 3 (20%) subjects with ventricular septal defects (VSDs), and 1 (6.67%) subject with Tetralogy of Fallot (TOF) were noted. Three subjects (20%) had either mitral valve prolapse (MVP), mitral stenosis (MS), or patent ductus arteriosus (PDA), or in combination.

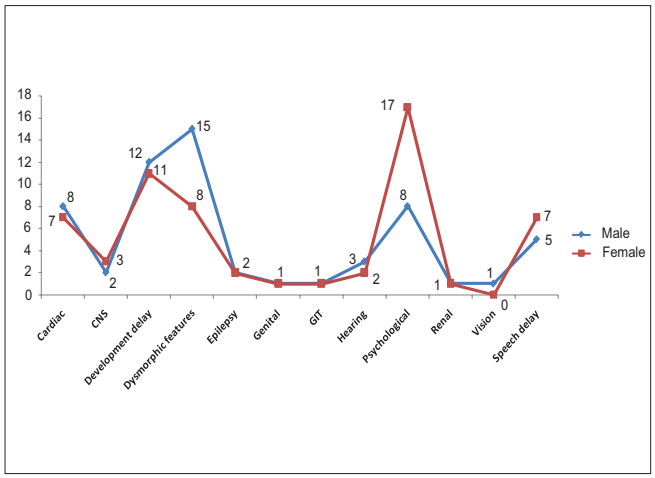

Figure 1: Distribution of other congenital anomalies among subjects

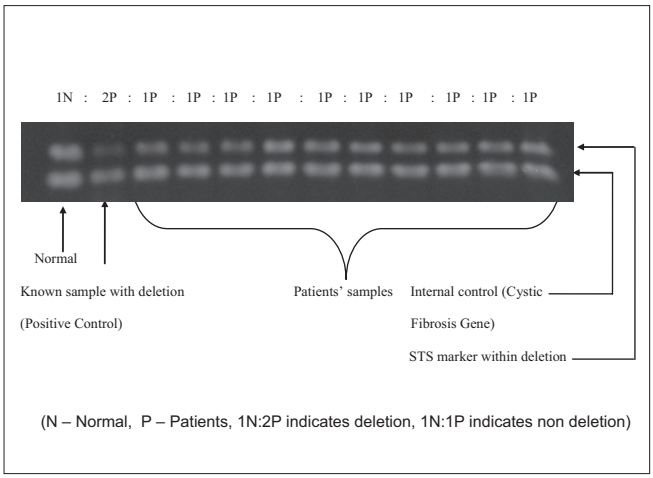

Figure 2: Dosage estimation of PCR products on 0.8% agarose gel (N = normal; P = patients; 1N:2P indicates deletion, 1N:1P indicates non-deletion)

Dysmorphic features

Dysmorphic features include abnormal facial features in 11 (47.83%) subjects, limb deformity in 6 (26.09%), and other minor abnormalities in another 6 (26.09%) subjects.

Developmental delay

Developmental delay including learning disability in 10 (43.48%), mild developmental delay in 9 (39.13%), and global developmental delay in 4 (17.39%) subjects was identified.

Psychological problem

Psychological problems were analyzed separately and were found in 25 (15.43%) subjects with cleft palate. Of these, 17 (68%) were females and 8 (32%) were males. Fear to talk in the public was the commonest presentation and was seen in 17 (68%) subjects. Aggressive behavior in 4 (16%) and other minor psychological problems in 4 (16%) subjects were also identified.

Identification of 22q11.2 microdeletion in patients with cleft palate by PCR

A total of 162 patients with cleft palate were investigated by quantitative multiplex PCR for STS markers spanning the 22q11.2 region. All PCR products were analyzed after agarose gel electrophoresis by using gel documentation system (Bio-Doc). There were no cases with 22q11.2 microdeletion identified [Figure 2].

DISCUSSION

Out of 323 subjects, 162 (50.15%) attended the clinic and participated in the study. Most of the patients had completed their surgical intervention, while few of them were waiting for their surgery.

Most of the patients (90.12%) were from the Southern Province of Sri Lanka. Majority (51.51%) were from Galle district while 24.78% were from Matara and 14.2% were from Hambantota districts. Patients from other districts including Kalutara (4.32%), Colombo (3.08%), and Ratnapura (2.48%) also participated in the study [Table 2].

In this group of 162 subjects with cleft palate, there were 95 (58.64%) females and 67 (41.36%) males. It shows that the cleft palate is more common in females than males in Sri Lanka. Chuangsuwanich *et al.*[25] examined 593 patients with cleft lip and palate in Thailand and found female predominance in the cleft palate group. Cleft palate is more common in females in china,[26] Australia,[8] and Glasgow.[27] In a study of 477 cleft palate patients in Jordan, Al-Omari and Al-Omari found that 74% of them were females, confirming the gender variation in cleft palate.[28] In Estonia also, a study on epidemiologic factors causing cleft lip and palate shows that there is a female predominance in the occurrence of cleft palate.[29] Results of all studies mentioned above are compatible with the results of the current study. Involvement of genetic factors such as X-linked recessive inheritance pattern has been explained by Rushton *et al.*[30] However, this is not in agreement with the results of above-mentioned studies as X-linked recessive inheritance is seen in male patients while females are only carriers.[30]

According to Hodgkinsons *et al.* (2005) in Northern Ireland, cleft in the secondary palate is commoner than the cleft in the primary palate.[2] In Brazil, cleft soft palate is commoner (80%) than complete cleft palate.[31] According to the present study, the cleft soft palate is the commonest and there were only lesser number of patients with complete cleft involving the entire secondary palate and primary palate. These findings are found compatible with the results from Brazil and Northern Ireland.[2,31]

Cleft palate is associated with lot of other major clinical anomalies. The published data vary significantly between studies. According to the available data, incidence of other associated anomalies varies from 2 to 55% worldwide.[26,27,32] Survey of patients with cleft lip and palate in China shows lesser number of cleft palate patients (2.18%) associated with other clinical manifestations.[26] An epidemiologic study of oral clefts in Iran showed 7.73% of cleft patients associated with other clinical manifestations, which is significantly higher when compared to the normal population.[32] In Bulgaria, Vera Krumova (2008) found that there were 43.3% of cleft palate patients associated with other clinical malformations.[29] According to the study of Boo *et al.* in 1990,

15.6% of cleft palate patients were associated with other clinical malformations, and in Estonia, 30.3% of patients with clefts had accompanying developmental anomalies.[33,34]

Data from the Glasgow Register of Congenital Malformations were used to investigate the epidemiology of congenital facial clefts over the period 1974–1985 by Womersley and Stone in 1987. They found more than half of the infants (54%) with isolated cleft palate had other associated defects and noted that these anomalies were common in female cleft palate patients than males (61%).[27] In Scotland, FitzPatrick *et al*.[35] identified that there was no significant association between gender and associated malformations in patients with cleft palate. This is not compatible with the results of this study where associated anomalies were common in males (54%) than females.

According to the Glasgow Register of Congenital Malformations, Pierre–Robin syndrome, musculoskeletal anomalies, neural tube defects, chromosomal abnormalities, and cardiovascular defects were the commonest defects associated with cleft palate.[27] In Denmark, congenital heart defects, Pierre–Robin syndrome, Down syndrome, mandibulofacial dysostosis, anal atresia, Turner syndrome, Hirshsprung' disease, and chromosomal anomalies were the common clinical features associated with cleft palate.[36]

Ruiter *et al*. in 2003 examined 99 patients with cleft palate and identified only one patient with 22q11 deletion among them and concluded that there is no justification for routine screening of 22q11 deletion in patients with cleft palate.[37] According to Driscoll,[38] the 22q11.2 deletion has not been found to be a cause of nonsyndromic cleft palate. Hence, prenatal testing is not recommended in the absence of other findings of 22q11 deletion syndrome. In this study, there were no patients found with 22q11 deletion among cleft palate subjects and it is compatible with the results of above-mentioned international studies.

CONCLUSION

Cleft soft palate is the commonest presentation of cleft palate and females are more prone to have cleft palate than males in Sri Lanka. Findings of this study further confirm the association of high incidence of congenital anomalies, developmental delays, dysmorphic features, and psychological problems in patients with cleft palate and reinforce the need of a high index of suspicion regarding the presence of such associated problems in cleft palate patients. Furthermore, it is advisable to search for syndromic diagnosis in patients with cleft palate. There is no justification for routine screening of patients with cleft palate for 22q11 deletion syndrome in Sri Lanka. It is advisable to formulate a guideline for screening of syndromic diagnosis and genetic investigation for cleft palate patients in Sri Lankan population.

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