RESEARCH PAPER

Clinical and Mutation Profile of Children with Cystic Fibrosis in Jammu and Kashmir

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Objective: To study the clinical and mutation profiles of children with cystic fibrosis in Jammu and Kashmir

Methods: One hundred consecutive patients presenting with one or more phenotypic features suggestive of cystic fibrosis (CF) were screened by quantitative sweat chloride testing. For patients with positive/equivocal test result on two occasions, CFTR gene mutation analysis was done by polymerase chain reaction.

Results: Of the 100 patients, 18 (10 females) were diagnosed to have CF at a median age of 10.5 y (IQR 4.75-15.25 y) while the median age at the onset of symptoms was 12 mo (IQR 4-63 mo) with a delay in diagnosis by 102.4±80.5 months. Clinical features at presentation included failure to thrive (94.4%), chronic cough (78%), recurrent pneumonia (61%), persistent pneumonia (11%), and chronic diarrhea (50%). Positive sweat chloride (>60 meq/L) was seen in 14 (14%) patients and 4 (4%) patients had equivocal (40-60 meq/L) value on two different occasions. Mutational analysis done in 15 patients showed DeltaF508 mutation in 20% (3/15) patients in homozygous form and in 13% (2/15) patients in heterozygous form. Intron 19 mutation 3849+10kb C>T was found in 40% (6/15) in heterozygous form. One (6.6%) patient had DeltaF508 and 3849+10kbC>T mutations in compound heterozygous form. Patients with equivocal sweat chloride and 3849+10kbC>T mutation had delayed onset of pulmonary involvement.

Conclusion: 3849 +10kbC>T mutation appears to be common in children with cystic fibrosis in Jammu and Kashmir followed by DeltaF508, although the data are quite limited. Although presentation is delayed and sweat chloride is in the equivocal range, severe lung involvement may occur in these patients.

Keywords: *CFTR, Cystic fibrosis, Mutations.*

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Cystic fibrosis (CF) is an inherited multisystem disorder of children and adults. Studies from Indian subcontinent suggest that CF is more common in people of Indian origin than previously recognized [1] but the precise incidence among Indians is not known. However, there is paucity of literature regarding the mutation profile in Indian children with cystic fibrosis as well as possible heterogeneity in different geographic regions [2-4]. Documentation of mutation profile from different regions may help in forming a common panel of mutations for each region. The present study was designed to look for the clinical features and mutation profile of children with cystic fibrosis in the state of Jammu and Kashmir.

METHODS

This study was conducted from November 2006 to October 2009 in the Pediatric unit of Sher-i-Kashmir Institute of Medical Sciences, a tertiary care hospital in Northern India. One hundred patients aged 1 month-18 years, deemed to be high risk for having cystic fibrosis were recruited for the study. These patients were recruited from the outpatient clinical setting and were admitted only if the circumstances demanded. These patients presented with clinical involvement of one or more organs with symptoms related to respiratory system – recurrent wheezing, recurrent/persistent pneumonia, chronic cough with or without sputum production, gastrointestinal tract – chronic diarrhea/steatorrhea, rectal prolapse, meconium ileus and or failure to thrive.

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The study was approved by the institutional review committee. In addition, consent was taken from the parents/patients before each child was included in the study.

All the patients were subjected to detailed evaluation with respect to history, clinical examination and baseline investigations, including chest radiograph. In addition,

other investigations like high resolution chest tomography (HRCT), microbiological evaluation of respiratory secretions and fecal fat analysis were also done. Pulmonary function testing was done by spirometery at admission and as and when needed in patients who were more than five years of age. Sweat chloride estimation was conducted in all the patients. Sweat was collected based on pilocarpine iontophoresis by Gibson and Cooke method [5] followed by estimation of chloride in the collected sweat by Schales and Schales method [6]. The iontophoresis was done by indigenously prepared and validated equipment by Kabra, *et al.* [7]. A minimum of 100 mg of sweat was collected for reliable results. Sweat chloride test was done on two occasions at least one week apart for patients with positive/equivocal results and in patients with negative results but with symptoms highly suggestive of CF.

The patients with positive or equivocal sweat chloride test on two separate occasions were subject to *CFTR* mutation analysis. The mutation analysis was done at the Genetics Unit, Department of Pediatrics of All India Institute of Medical Sciences (AIIMS), New Delhi. For detection of mutation, 3-5 ml of blood was collected in EDTA vaccutainer. DNA was extracted from blood leukocytes as per the standard procedures for molecular genetic analysis of the *CFTR* gene [4]. The mutations for which screening was done included DeltaF508 (HGVS nomenclature, c.1521_1523delCTT) and 3849+10kbC>T (c.3718-2477C>T) mutations. We could not screen for all the mutations seen in the Indian subcontinent because of the high cost involved in the analysis for each mutation. We selected DeltaF508 and 3849+10kbC>T as these were common mutations seen in few children from Jammu and Kashmir who were diagnosed at AIIMS before this study. Only two patients with suggestive symptoms had inadequate sweat production, and were negative for both the mutations tested.

RESULTS

Out of 100 high risk patients (60 males), 18 (8 males) had cystic fibrosis diagnosed either with mutation analysis and/or sweat chloride estimation and constituted the study group. The median age was 10.5 years (IQR 4.7-15.2 years). Age at presentation was 127.5±95.3 months while age at onset of symptoms was 25.1±30.3 months.

A significant delay in diagnosis of the CF patients with a mean±SD age of 102±80.5 months was documented. Consanguinity was seen in 10 (55%) patients and history of CF like illness in sibs who had died of disease profile similar to CF was present in 8 (44%) patients.

Presenting clinical features are summarized in ***Table* I**. Abnormal clinical chest examination (83%) consisted of either hyperinflation or loss of lung volume secondary to bronchiectasis. Since majority of our patients were diagnosed late, bronchiectasis with loss of lung volume was seen more in our patients than hyperinflation. One 18 year old male diagnosed with cystic fibrosis had presented with azoospermia and congenital bilateral absence of vas deferens (CBAVD) diagnosed by absence of vas deferens on ultrasound.

Radiological findings are shown in ***Table* II**. In two infants diagnosed with CF in first six months of life, HRCT chest showed only air trapping and hyperinflation. Sputum culture, including cough throat swabs and one

TABLE I CLINICAL PROFILE OF CHILDREN WITH CYSTIC FIBROSIS (N=18)

Clinical presentation	*No. (%)*
Recurrent wheezing	10 (55.6%)
Chronic cough with sputum	14 (77.8%)
Hemoptysis	3 (16.7%)
Recurrent pneumonia	11 (61%)
Persistent pneumonia	2 (11%)
Recurrent sinusitis	5 (28%)
Failure to thrive (Malnutrition)	17 (94%)
Steatorrhea/Chronic diarrhea	9 (50%)
History of meconium ileus	3 (16.7%)
Rectal prolapse	1 (5.6%)
Digital clubbing	13 (72%)
Abnormal chest examination	15 (83%)

TABLE II RADIOLOGICAL FEATURES IN PATIENTS WITH CYSTIC FIBROSIS (N=18)

Radiological Feature	*No. (%)*
CXR	
Consolidation	8 (44%)
Honey combing	10 (55.6%)
Perihilar Infiltrates	11 (61%)
Prominent bronchovascular markings	6 (33%)
Hyperinflation	7 (39%)
CT-Chest	
Diffuse bronchiectasis	11 (65%)
Lobar bronchiectasis	3 (17.6%)
Consolidation	8 (47%)
Cavity	2 (12%)
Air trapping, mucous plugging	12 (70.6%)

tracheal aspirate revealed growth of *Pseudomonas aeuroginosa* in 9 (53%) patients and *Staphylococcus aureus* in 1 (6%) patient. No patient underwent bronchoscopy.

Mutation analysis: Out of 18 CF patients, *CFTR* mutation analysis could not be done in three patients. Two patients were lost to follow up, and one infant who presented with respiratory failure died soon after diagnosis. In this patient, the diagnosis of cystic fibrosis was made by sweat chloride estimation. DeltaF508 was found in 3 (20%) patients in homozygous form and in 2 (13.3%) patients in heterozygous form. Intron 19 mutation 3849+10kbC>T was found in 6 (40%) in heterozygous form. One (6.6%) patient had DeltaF508 and 3849+10kbC>T mutations in compound heterozygous form. Out of the total seven patients with 3849+10kbC>T mutation, four patients had sweat chloride in the equivocal range (including one patient with 3849+10kbC>T mutation and DeltaF508 mutation in heterozygous state), and three patients had sweat chloride >60 mEq/L. ***Table* III** compares the clinical features of patients with DeltaF508 mutation and 3849+10kbC>T mutation. Three out of four patients with 3849+10kbC>T mutation had normal/equivocal sweat chloride levels and delayed onset of pulmonary disease (median 48 months).

DISCUSSION

Cystic fibrosis is considered to be uncommon in state of Jammu and Kashmir in India. Contrary to this belief, our study suggests that CF is not uncommon in our population and the genetic profile may possibly be different from rest of the country.

CF was diagnosed in 18% of high risk patients in our study. In a study by Kabra, *et al.* [3], CF was detected in 120 (3.5%) of all children attending their pediatric chest clinic over a period of 7 years. Other reports have also been published [8,9]. The median age at diagnosis was 10.5 years with significant delay in diagnosis. This is in contrast to US where 71% of CF cases are diagnosed by first year of life [10]. This shows the low index of suspicion for the disease in our society and need for increased awareness for same.

Carrier frequency of DeltaF508 mutation in Indian population is estimated as 0.42% and gene frequency as 0.21%. Frequency of cystic fibrosis patients with homozygous DeltaF508 mutations is 1/ 228006 and the estimated prevalence of cystic fibrosis is 1/43321 to 1/100323 in Indian population [11]. Our study showed DeltaF508 mutation in 27% of CF patients.

The patients having 3849+10kbC>T mutation in both homozygous and heterozygous form had pulmonary involvement with median age at diagnosis of 12 years. Except for one female patient with symptoms starting in early infancy, the other six patients had history suggestive of CF symptoms starting in later part of the first decade of life. The delay in diagnosis was both because of low index of suspicion for the disease and late pulmonary presentation. However, age-adjusted severity of lung disease and function was comparable to the patients with DeltaF508 mutation. Similar presentation has been reported by Stern, *et al.* [12] and Augerten, *et al*. [13]. 3849+10kbC>T mutation is a relatively uncommon *CFTR* gene mutation with an overall frequency of 1-2% and an elevated prevalence in individuals of Ashkenazi Jewish ancestry [19]. This mutation is associated with a mild form of CF [13,15]. However, a marked variability in disease severity is found among patients with this allele, and several have a severe pulmonary disease [16,17].

Four patients in our study had sweat chloride concentration in the intermediate range (40-60 mmol/L). Such cases are now labelled as cystic fibrosis transmembrane conductance regulator related metabolic syndrome (CRMS) which is proposed to describe infants who have sweat chloride values <60 mmol/L and up to two *CFTR* mutations, at least one of which is not clearly categorized as a 'CF-causing mutation', thus they do not meet CF Foundation guidelines for the diagnosis of CF [18]. These patients are more likely to be pancreatic

TABLE III COMPARISON OF CF PATIENTS WITH DELTA 508 AND 3849+10KB C>T MUTATION

Mutation	*Delta 508 (n=6)*	*3849+10kb C> T (n=7)*
Age, Median (IQR), y	10.5	12.0
Age at onset of symptoms, (Median IQR), mo	9.0	48.0
Female sex (%)	5 (83.3%)	5 (71.4%)
CF like illness in sibling (%)	3 (50%),	1 (14.3%)
Consanguineous marriage (%)	3 (50%)	4 (57.1%)
Sweat chloride mmol/L (mean±SD)	95±46.6	63.9±14.9
Growth Failure N (%)	5 (83.3%)	7 (100%)
Pulmonary invovement N (%)	6 (100%)	7 (100%)
GI invovement N (%)	6 (100%)	4 (57.14%)
Hemoptysis N (%)	0	3 (42.85%)
Rectal prolapse N (%)	1 (16.7%)	0
Diffuse bronchiectasis N (%)	4 (66.7%)	5 (71.4%)
Lobar bronchiectasis N(%)	1 (16.7%)	2 (28.6%)

One patent having compound heterozygous mutation DF508/ 3849+10Kb C>T figures in statistics in both mutations

WHAT IS ALREADY KNOWN?

- Although several mutations are seen in Indian children with cystic fibrosis, there is paucity of literature regarding mutations in children with cystic fibrosis in Jammu and Kashmir.

WHAT THIS STUDY ADDS?

- This study gives the phenotypic and genotypic correlation of children with cystic fibrosis in Jammu & Kashmir.

sufficient as assessed by fecal elastase measurement. CRMS patients may develop signs of cystic fibrosis but usually have a milder clinical course than patients with CF. These patients may have a different prognosis from patients with 'classic CF,' but some develop progressive lung disease as a result of chronic airway infection [19-21].

The strength of this study is that this is the first study in children with cystic fibrosis in Jammu and Kashmir where phenotypic features have been correlated with genetic mutations. However, the study had many limitations. Only 18 subjects could be diagnosed over three years and genetic study for mutations could be done in only 15 patients. The other limitation is that because of financial constraints screening could not be done for all the mutations seen in the Indian subcontinent.

In conclusion, 3849+10kbC>T appears to be a common mutation followed by DeltaF508 in children with cystic fibrosis in the study population although the data is quite limited. Although presentation is delayed and sweat chloride is in the equivocal range, severe lung involvement can occur in these patients. Therefore, in suspected patients *CFTR* mutation testing should be done if sweat chloride is in the equivocal range. Studies with large number of subjects are needed to confirm these findings.

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