Cystic fibrosis detection in high-risk Egyptian children and CFTR mutation analysis

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Abstract

Background: Knowledge about Cystic Fibrosis (CF) in Egypt is very limited. The objective of this study was to screen for CF in Egyptian children with suggestive clinical features and to identify causative genetic mutations.

Methods: Sixty-one patients from the Chest Unit, Cairo University Children’s Hospital, Egypt, were included. Subjects presented with persistent or recurrent respiratory symptoms, failure to thrive, diarrhea and/or steatorrhea and unexplained persistent jaundice. Patients were screened using the CF Indicator™ sweat test system (PolyChrome Medical, Inc., Brooklyn Center, MN). A quantitative sweat testing was conducted on 10 of the 12 positive patients. Seven probands and one sibling underwent molecular analysis by direct DNA sequencing of the coding region and of the intronic sequences adjacent to the 27 exons of the CFTR gene.

Results: Of 61 patients, 12 (20%) had positive sweat chloride screening. Ten of the 12 patients underwent quantitative sweat testing and were positive. Eight CFTR sequence changes were identified in seven affected probands and two were confirmed in one sibling by direct DNA sequencing.

Conclusion: The study results suggest that CF is more common in Egypt than previously anticipated. Larger studies are warranted to identify the incidence, molecular basis and clinical pattern of CF in the Egyptian population.

Keywords: Cystic fibrosis screening; CFTR; Non-Caucasian CF; CF indicator™ sweat test system; CF in Egyptian children

1. Introduction

Cystic fibrosis (CF) is a chronic, progressive, recessively transmitted genetic disorder [1]. It is considered the most common life-limiting genetic disease in the Caucasian population [2]. In the United States (U.S.), CF incidence in Caucasian populations is ranging from 1 in 1900 to 1 in 3700 [3]. It is lower among African Americans (1 in 15,000) [3,4] and Asians (1 in 32,000) [5,6]. However, the incidence in populations of other ethnic backgrounds, including Egyptians, has not been established [3].

The median age of diagnosis in the U.S. is 6 months and the mean is 3.3 years. Survival of CF patients has shown steady improvement, as documented in the U.S. Patient Registry [4]. As a result of specialized care provided through accredited centers, the predicted median survival of CF patients currently is 35.1 years [4]. Improvement in the
median age of survival is due to advances in care and to the availability of new medications for CF (e.g. tobramycin solution for inhalation and recombinant human DNase) [7]. Delayed diagnosis could lead to irreversibly progressive pulmonary disease and a shortened life span [8].

Minimal information is available concerning the prevalence of CF in Egypt, where the disease was presumed to be extremely rare. A few studies of CF patients from the Middle East have described the clinical presentation and limited genetic analyses of the disease [9–19]. Many children with CF in these populations probably remain undiagnosed due to lack of clinical suspicion and proper diagnostic facilities. The objective of this study was to determine the presence of CF among 61 Egyptian patients with respiratory and gastrointestinal manifestations similar to those reported in CF patients [2]. The children were seen at Cairo University Children’s Hospital. A description of their clinical findings and their molecular genetic analysis will be presented. This research study was a collaboration among Cairo University, University of Michigan Health System, Cystic Fibrosis Center, Ann Arbor, MI and Stanford University Medical Center, Stanford, CA.

2. Materials and methods

2.1. Subjects

Sixty patients aged 2 months to 2 1/2 years were screened from Cairo University Children’s Hospital—a tertiary care referral hospital of 300 beds located in Egypt. Subjects were recruited during a 13 month period (November 2000–December 2001). High risk patients were selected from the pulmonology and the gastroenterology outpatient clinics and inpatient wards. Subjects presented with one or more of the following symptoms were asked to participate in the study: chronic or recurrent respiratory symptoms, chronic cough, recurrent pneumonia and/or persistent radiographic abnormalities, gastrointestinal symptoms such as diarrhea, steatorrhea, rectal prolapse, meconium ileus or hepatobiliary disease, and/or failure to thrive with no other diagnosis to explain their clinical condition. A seven-year-old boy was screened because he presented with symptoms similar to his sibling, who tested positive for CF in the screening stage. These two siblings were the only related subjects in the study.

2.2. Study methods

2.2.1. Qualitative sweat testing

Sixty-one patients were screened for CF using the Model 9800 CF Indicator™ Sweat Test System (Polychrome Medical (PCM) Inc., Brooklyn Center, Minnesota). This is a simple, rapid, laboratory-independent sweat test [20–23]. The portable test unit uses pilocarpine iontophoresis for sweat stimulation followed by placement of a chloride concentration test patch to collect and analyze the sweat. The assay provides a qualitative assessment of the sweat chloride concentration. Results are interpreted based on color change of the test patch and categorized as follows: CF not present (negative test), CF possible (test is inconclusive) or CF present (positive test denoting sweat chloride >60 mEq/L). The CF Indicator™ sweat test was compared to the Gibson–Cooke quantitative sweat test [24]. The specificity were 98.9% and 100% and sensitivity were 100% and 100% for the CF Indicator and Gibson–Cooke sweat test, respectively [22]. Twelve patients of the 61 tested were positive by this screen. Patients with repeated inconclusive test results were considered negative for CF (Group B). Close follow-up of this group of patients is ongoing with further laboratory re-evaluation as necessary.

2.2.2. Quantitative sweat testing

Ten of the 12 CF-positive patients were available for quantitative assessment of sweat chloride for confirmation of the diagnosis. Sweat gland secretion was stimulated using the pilocarpine iontophoresis method followed by sample collection [24]. The chloride level in collected samples was subsequently measured. At least 50 mg of sweat was collected for reliable results. Sweat chloride analysis was repeated twice for all tested patients to confirm the results. An average of the two readings is shown in Table 2.

2.2.3. PCR amplification

Eight of the twelve positive patients were genetically tested. Genomic DNA was isolated from blood leukocytes according to standard procedures for the molecular genetic analysis of the CFTR gene. Amplification of genomic DNA was performed using Amplitaq Gold DNA polymerase, 5 U/μL (Perkin Elmer) and the four 2’-deoxynucleotide 5’-triphosphates (Amersham Pharmacia). Primer pairs from intronic sequences that flank individual CFTR exons were used for amplification of the CFTR gene as described [25]. For exon 1, however, an alternative forward primer was substituted [26]. The presence and size of amplified products were confirmed by agarose-gel electrophoresis.

2.2.4. CFTR sequencing analysis

PCR purification was performed by QIAquick PCR purification or by QIAquick gel extraction (Qiagen). Purified samples were sequenced directly with fluorescent di-deoxy terminators (ABI) on an ABI 310 or 377 sequencing instrument (Applied Biosystems). Sequence interpretation was facilitated by GCG Sequence Analysis Web alignment programs (www.Accelrys.com). All identified sequence changes were confirmed by direct DNA sequencing in the reverse direction.

3. Results

The study population consisted of 61 patients. We initially screened 60 patients ranging in age from 2 months to 2 1/2 years with a mean age of (±SD) 1.1±0.68 years.
There were 41 boys (age 1.1±0.71) and 19 girls (age 1.2±0.63). Patients were divided into two groups based on the results of sweat chloride testing. Group A consisted of 11 patients (18%) with a positive screening test and Group B encompassed 49 patients (82%) with negative screening results. Demographics and clinical presentation of the study population are illustrated in Table 1. A seven-year-old patient was included in the screening process after his 2 1/2-year-old sibling tested positive for CF (Table 2).

A high rate of consanguineous marriage was documented in the families of the study population, with no significant difference between the two groups (Group A: 84% and Group B: 82%). Eleven patients in Group A (92%) and 41 patients in Group B (84%) were breast fed. None of the patients had nasal polyps or history of meconium ileus.

Table 2 presents clinical characteristics and sequence analysis of 12 CF positive patients from 11 unrelated families. In patients with CF the principal clinical features were growth failure (weight and length below 5th percentile for age) and persistent respiratory symptoms. Diarrhea was frequently described rather than steatorrhea, and one patient developed rectal prolapse during follow-up. One patient presented with jaundice, impaired liver functions and growth failure with no pulmonary signs or symptoms. However, jaundice and hepatomegaly were more frequent in Group B than Group A (26.5% and 14.3% respectively). Ten of the 12 CF positive patients using qualitative sweat testing were also assessed by quantitative sweat testing. Sweat chloride concentrations for the ten patients ranged from 80–120 mmol/l with a mean (±SD) of 94.3±10.8. One patient (#58) was not available for either quantitative sweat testing or sequence analysis. He underwent extensive work up for other causes for his symptoms, with negative results. Because his symptoms were consistent with CF and he was positive by the qualitative sweat testing, he was considered positive for CF.

3.1. Mutation analysis

Eight of the 12 CF-positive patients were genetically tested. Amplification and direct genomic DNA sequencing of the 27 CFTR exons in the seven probands and one sibling revealed a total of eight mutations (Table 2). The ΔF508
mutation was present three times in the study population: In a homozygous patient whose affected sibling had the same mutation and also in a patient for whom the second mutation remained unidentified.

One patient was compound heterozygous for 1898+3A>C and 4041G>C. The 1898+3A>C mutation is likely to result in a mRNA splicing defect since it occurs at the donor splice site of intervening sequence 12. It was originally identified on a Moroccan chromosome (Cystic Fibrosis Mutation database http://www.genet.sickkids.on.ca/cftr/). This mutation is rare in Caucasians and does not reach the 0.1% threshold of general population frequency to warrant screening in the U.S. [27]. The 4041G>C mutation results in an amino acid substitution of an asparagine residue by lysine at position 1303 (N1303K). This mutation is relatively common in the Caucasian population and is included in the ACMG/ACOG recommended carrier screening panels.

One patient was identified as a carrier of the 2125A>T (T665S) missense mutation in exon 13a. This rare mutation was originally identified in a Tunisian CF patient (Cystic Fibrosis Mutation database http://www.genet.sickkids.on.ca/cftr/) and is located close to an exonic splice enhancer (ESE). The mutation may potentially cause aberrant splicing of exon 13 by influencing the polypyrimidine tracts of two cryptic splice sites [28].

Finally, the 1540A>G (M470V) sequence variant was detected in two patients in whom no clearly pathogenic mutations were identified. Although this change is described as a sequence variant based on its relative frequency (Cystic Fibrosis Mutation database http://www.genet.sickkids.on.ca/cftr/), it has been associated with clinical significance when found in combination with the 5T allele in intron eight [28]. Although our two patients were homozygous for the common 7T allele in intron eight, it is conceivable that the M470V amino acid substitution results in a minor contribution to the development of the phenotypic spectrum of CF [29–31]. The frequency of the M470V allele in the Egyptian population is not known. Considering its common occurrence in other populations, however, it is not likely to significantly contribute to the phenotype of these patients.

4. Discussion

A diagnosis of CF is considered when there are characteristic symptoms, a history of CF in a sibling, or a positive newborn screening test, in addition to a positive sweat test or the detection of two mutations in the CFTR [5]. Cystic fibrosis has been believed to occur infrequently in Egypt, however the present study suggests that the occurrence of CF in Egyptian patients is higher than previously thought. In this small group of high-risk patients, CF diagnosis was made in 20% of subjects. In a previous study, neonatal screening for CF was conducted for 18,650 Egyptian infants, using the meconium BM-mec-test (Boehringer Manheim, Gmbh, Germany). Five infants were identified as positive for CF and four were borderline. Subsequent qualitative sweat testing indicated seven of the nine babies were positive for CF with an incidence rate of 1:2664 [10]. Two hundred twenty-four patients age 3 days to 7 years were also tested using the same qualitative sweat chloride testing method (the Orion’s Model 417 Skin Chloride System). The sweat test was considered positive if the sweat chloride level was greater than 60 mM/L. Four of these patients tested positive (1.79%) [10]. Neither study evaluated for the presence or absence of CF-related symptoms. Few reports have been published about CF patients in the Middle East [11–19]. These reports indicated frequencies ranging from 1:5800 in Bahrain [15] to 1:2650 in Jordan [16].

In the majority of cases (71%) in the United States, the diagnosis of CF is established by the age of 1 year [2,4]. It is likely that a delayed diagnosis contributes significantly to the high prevalence of malnutrition in CF patients [32,33] and could also lead to irreversibly progressive pulmonary disease and a shortened life span [8]. The patients who were diagnosed with CF in our study have been monitored closely. Appropriate pancreatic supplements, in addition to aggressive treatment plans for their respiratory and GI symptoms, have been started with positive preliminary results.

The lack of definitive data for the prevalence of CF in Egypt may reflect underdiagnosis of CF where a significant proportion of infant morbidity and mortality is caused by respiratory infections, diarrheal disease and malnutrition [8,9]. Another confounding factor may be early mortality caused by severe disease or neonatal complications. In addition, lack of awareness of the public and medical community in Egypt, concerning the presentation and diagnosis of the disease may be a contributing factor to underdiagnosis of the disease.

The clinical presentations of CF are heterogeneous. The frequency of clinical manifestations is better described in populations where the disease is well studied [4]. Due to genetic and environmental differences among ethnic groups, CF presentation may also vary between populations. In the present study, all CF patients had failure to thrive at the time of diagnosis, 92% had chronic or recurrent respiratory disease and 58% had an abnormal stool pattern described as watery diarrhea, as opposed to “steatorrhea” which is more commonly associated with CF [4]. In contrast, reports from the North American Cystic Fibrosis Registry [4,5] indicate that 40.3% of CF patients had failure to thrive, 48.8% had respiratory symptoms and 32.2% had abnormal stools/steatorrhea at time of diagnosis. The present study, however, did select high-risk patients with chronic illness and findings may be influenced by a delay in diagnosis or indicate a more severe disease presentation. Clinical phenotypes reported in studies from other populations in the Middle East suggest a relatively high incidence of hepatobiliary manifestations in CF. Hepatobiliary involvement was reported in 4% [15] to 10.9% [11,12] of Middle Eastern patients diagnosed with CF; in the present study one patient presented primarily with
hepatobiliary problems. This frequency of hepatobiliary involvement is much higher than that reported in the North American CF Registry [4,5]. Although the studies are diverse and the populations studied were small in number, the findings may represent a different spectrum of CF manifestations in the Middle East.

In our patient population there were more males than females (67% in both groups) (Table 1). This finding could reflect a true increase of disease incidence in males or may suggest more severe disease in females with early demise. We are not aware of gender-specific referral practices that may have influenced such results.

The study also revealed a high rate of consanguineous marriage (84% in Group A, 82% in Group B), higher than the reported rate for the general population in Egypt (37%) [34]. This finding is not surprising, given that the study specifically included high risk patients with possible genetic disorders including CF. Twenty-three percent of our CF patients had a suggestive family history, which included death of siblings with undiagnosed conditions and similar symptoms.

The AF508 mutation is the most common mutation in Caucasians, where it accounts for approximately 70% of CF cases. Reports on CFTR genetic analysis in Middle Eastern populations have reflected genetic mutations that are distinct from populations of Northern European descent and putative novel mutations (e.g. del E672 and IVS21-28G>A) have been identified [11,33]. In our 7 probands, we expected to identify 14 mutations, given the autosomal recessive inheritance pattern of CF. However, only 8/14 mutant alleles were identified (57%). Direct DNA sequencing has a very high detection rate (~99%) for sequence changes in the CFTR coding region and adjacent splice sites, but is unable to detect large deletions, mutations in the intervening and other non-coding sequences, or variants in the promoter. Thus, it is a possibility that mutations which would escape detection by sequencing are common in the Egyptian population, or at the least in this group of patients. Further studies need to be conducted to test this hypothesis.

In conclusion, this study reveals the need for further investigation of cystic fibrosis in Egyptian children at an early age. Previously thought to be extremely rare, the true incidence of the disease in Egypt needs to be established. Its true frequency may be obscured by more common conditions such as respiratory infections, diarrhea and malnutrition as well as other genetic or metabolic diseases. The current study was limited by the small number of patients and by the lack of implementation of more standardized diagnostic measures such as quantitative sweat chloride testing [24]. Further analysis of the clinical manifestations of the disease in Egyptian children and characterization of the mutation spectrum are therefore warranted. Finally, establishment of a CF care program to provide diagnosed patients with specific treatment plans is necessary to improve the quality of life and to prolong patient survival. As part of this effort, the molecular basis of CF should be characterized in a larger group of Egyptian patients in order to identify common mutations in this population and to provide confirmation of the clinical diagnosis.

References