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Profile of cystic fibrosis in a single referral center in Egypt



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ABSTRACT

It was generally believed that Cystic fibrosis (CF) is rare among Arabs; however, the few studies available from Egypt and other Arabic countries suggested the presence of many undiagnosed patients. The aim of the present study was to determine the frequency of CF patients out of the referred cases in a single referral hospital in Egypt. A total of 100 patients clinically suspected of having CF were recruited from the CF clinic of the Allergy and Pulmonology Unit, Children's Hospital, Cairo University, Egypt, throughout a 2 year period. Sweat chloride testing was done for all patients using the Wescor macroduct system for collection of sweat. Quantitative analysis for chloride was then done by the thiocyanate colorimetric method. Patients positive for sweat chloride (≥ 60 mmol/L) were tested for the ΔF508 mutation using primer specific PCR for cystic fibrosis transmembrane conductance regulator (CFTR) gene. Thirty-six patients (36%) had a positive sweat chloride test. The main clinical presentations in patients were chronic cough in 32 (88.9%), failure to thrive in 27 (75%), steatorrhea in 24 (66.7%), and hepatobiliary involvement in 5 (13.9%). Positive consanguinity was reported in 50% of CF patients. Thirty-two patients were screened for ΔF508 mutation. Positive ΔF508 mutation was detected in 22 (68.8%) patients, 8 (25%) were homozygous, 14 (43.8%) were heterozygous, and 10 (31.3%) tested were negative. CF was diagnosed in more than third of patients suspected of having the disease on clinical grounds. This high frequency of CF among referred patients indicates that a high index of suspicion and an increasing availability of diagnostic tests lead to the identification of a higher number of affected individuals.

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Introduction

CF is the most common potentially lethal and life-shortening genetic diseases among populations of white Caucasian des-

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cent, such as those of Europe, North America, and Australia, being caused by mutations of the (CFTR) gene [1]. The incidence of CF varies according to the ethnic origin, ranging from one in 2000 to one in 3500 Caucasians born in Europe, the United States, and Canada [2].

Although extensively studied, the pathophysiology of CF remains a challenge for scientists and clinicians. Clearly, the detection of the causative gene (CFTR) and its predominant mutation (delta F508) was a milestone in the CF research. Since then, more than 1800 other mutations in the CFTR genes were detected [3].

Disruption of CFTR function has distinct consequences for different parts of the body, and certain organs seem to be more sensitive than others [4]. Although CFTR expression is found in the airway, salivary glands, pancreas, liver, sweat ducts, and reproductive tract, it is the impact on the lungs and the gastrointestinal tract that has the major consequence for morbidity and mortality [5,6]. Early diagnosis and advances in the care of CF patients has improved survival, and as a result, patients with the disease often live beyond the third decade [7,8].

Limited data are available regarding CF prevalence among Egyptian children. CF has been believed to occur infrequently in Egypt; only few papers suggested its presence [9,10]. The clinical expression of the disease and the degree of involvement of different systems (respiratory, gastrointestinal, reproductive, etc.) may vary in different populations and in children of variable racial descent.

Therefore, the aim of the present study was to detect the frequency of patients diagnosed as CF among patients clinically suspected of having the disease and referred to Allergy and Pulmonology Unit, Children's Hospital, Cairo University, Egypt, through a period of 2 years and to detect the frequency of Delta F508 mutation among those diagnosed as CF.

Patients and methods

This is a longitudinal study recruiting patients clinically suspected of having CF and referred to the CF clinic of the Allergy and Pulmonology Unit, Children's Hospital, Cairo University, Egypt, throughout a 2 year period from February 2010 to February 2012. *The study was approved by the scientific research committee of the Pediatric Department, Faculty of Medicine, Cairo University and a written consent was obtained from all parents after they were fully informed of the details.*

Patients included in this study had manifestations that suggested the diagnosis of CF such as respiratory manifestations, including chronic productive cough, bronchiectasis, recurrent pneumonia, hemoptysis, recurrent sinusitis, nasal polyps, clubbing, and/or gastrointestinal manifestations as meconium ileus in neonates, malabsorption, steatorrhea, and/or failure to thrive or short stature.

Sweat chloride test was done for all patients included in the study using the standard pilocarpine iontophoresis for sweat induction and the Wescor macroduct system for sweat collection as recommended by the NCCLS and cystic fibrosis Foundation (CFF) guidelines [11,12], followed by quantitative analysis of the collected sample. Sweat test was repeated for those who had a positive or equivocal sweat test results. The delta F 508 mutation was done for patients who had positive sweat test results.

Other routine investigations were done including complete blood picture, stool analysis, sputum culture, plain x-ray, and computerized tomography of the chest. The non-CF patients were further investigated to reach final diagnosis as follows: bronchopulmonary dysplasia, immotile cilia syndrome, alpha-1-antitrypsin deficiency, immunodeficiency, tuberculosis and congenital bronchiectasis, celiac disease, and food allergic enteropathy.

Quantitative sweat chloride testing

Sweat stimulation was done using the pilocarpine iontophoresis and sweat collection by the Wescor macroduct sweat

collection system [11]. The sweat sample was analyzed quantitatively by the thiocyanate colorimetric method. The average total volume of sweat sample is 20–50 ul. The Chloride was assayed colorimetrically based upon the competition of Hg^{2+} and Fe^{2+} for thiocyanate. The preferred Hg-thiocyanate adduct exhibits no color. In the presence of chloride, Hg^{2+} forms mercuric chloride freeing up thiocyanate, which then binds to the available Fe^{2+} exhibiting an absorbance at 450 nm. The intensity of the color is directly proportional to the chloride concentration in the sample. By using a Chloride standard with known concentration (100 mmol/L), the intensity of the color is converted to concentration according to Beer's Law. The Beer–Lambert law (or Beer's law) is the linear relationship between absorbance and concentration of an absorbing species. The general Beer–Lambert law: $A = a(\lambda) * b * c$, where A is the measured absorbance, $a(\lambda)$ is a wavelength-dependent absorptivity coefficient, b is the path length, and c is the analyte concentration. The intensity of the color formed is proportional to the chloride ion concentration in the sample [12].

Reference values of quantitative chloride analysis are as follows: <40 mmol/L = negative, 40–60 mmol/L = borderline/indeterminate, \geq 60 mmol/L = positive and consistent with the diagnosis of CF.

Molecular analysis

All patients with positive sweat chloride test were screened for the presence of Delta F508 gene mutation as follows:

DNA extraction

DNA was extracted from whole blood samples using Qiagen DNA extraction kit (QIAamp DNA mini kit; Qiagen, Hilden, Germany) and following the manufacturers' protocol.

PCR amplification

Screening for Delta F508 mutation was performed by Allele Specific Polymerase Chain Reaction (ASPCR) as previously described by Schwarz and Malone [13]. The following primers were used to detect the delta F508 mutation: forward normal, -5'-ggcaccattaaagaaaatcatctt-3', forward mutant-5'-ggcaccattaaagaaaatcattgg-3', and common reverse 5'-gttggcatgctttgatgacgcttc-3'. The PCR components were as follows: 10× Buffer without MgCl_2 , 50 mM MgCl_2 , 25 mM dNTPs, 5U/ul Dream Taq DNA polymerase (MBI Fermentas, Vilnius, Lithuania), 50–100 ng DNA, and 0.4 mM of each of the primers.

PCR reactions were performed using the thermal cycler PCR Express (Thermo Hybaid, Middlesex, UK). The final PCR volume was 25 ul. The amplification conditions were as follows: initial denaturation at 94 °C for 1 min (1 cycle) followed by 30 amplification cycles, denaturation at 94 °C for 1 min, annealing at 60 °C for 45 s, and extension at 72 °C for 1 min, with a final extension step at 72 °C for 6 min.

Ten microliters of amplification products were analyzed by means of vertical electrophoresis in 8% polyacrylamide gels for 90 min (120 V) using the Bio-Rad Mini-Protean tetra gel system (Bio-Rad, Hercules, CA, USA).

DNA samples of non-carrier subjects (having 2 wild-type alleles) yielded a unique 98 base-pair (bp) fragment, whereas

samples from heterozygous patients had 2 amplified fragments, 1 of 98 bp and 1 of 95 bp (lacking 3 base pairs), and finally, DNA from homozygous individuals had only 1 amplified fragment of 95 bp.

Statistical analysis

Results were analyzed using the Statistical Package for the Social Sciences program (SPSS) version 13.0.1 and the statistical program KyPlot version 3.0.2 h. Data were summarized using mean, standard deviation (SD) and range for quantitative variables and number and percent for qualitative variables.

Results

The present study enrolled 100 Egyptian children with clinical symptoms and signs suggestive of CF. Patient's age ranged from 2 months to 16 years with a mean age of 3.65 ± 3.87 years. Sixty-one of them were males and 39 were females. Basic demographic and clinical data of all patients included in this study are shown in Table 1, where the main clinical presentations of the study population were chronic cough in 84% of cases, failure to thrive in 56%, steatorrhea in 37%, and less frequently other presentations as clubbing, hepatobiliary system affection, sinusitis, and nasal polyps. Calculated sensitivity and specificity indicate the common clinical presentations of the referred population consistent with the diagnosis of CF.

Average age at diagnosis was 3.9 years. 22 (61.1%) of them were boys and 14 (38.9%) were girls. Being referral hospital, Patients came from various parts of Egypt, and Consanguinity was positive in 18 (50%) patients. History of definite CF in the family was present in 6 patients (16.7%). Basic demographic data of CF patients are shown in Table 2.

The main clinical presentations were chronic cough in 32 (88.9%), failure to thrive in 27 (75%), and steatorrhea in 24 (66.7%) patients. Details of clinical profile of CF patients are shown in Table 3.

The sweat chloride test was positive in 36 patients. The mean value of sweat chloride test in CF patients was 86.8 ± 14.7 mmol/L (mean \pm SD). Delta F508 mutation could be identified in 22 (61.1%) patients, 8 (22.2%) patients were homozygous, 14 (38.9%) were heterozygous, 10 patients were negative, and the test was not done for 4 (11.1%) patients as shown in Table 4.

Table 2 Demographic data of CF patients.

Variables	N (%)
<i>Age</i>	
Range	2 month–16 years
Mean \pm SD	3.91 \pm 4.18 years
<i>Gender</i>	
Male	22 (61.1%)
Female	14 (38.9%)
Male:female ratio	1.6:1
Positive family history of CF	6 (16.7%)
Positive consanguinity	18 (50%)
<i>Residence</i>	
Greater Cairo	22 (61.1%)
Delta	10 (27.8%)
Upper Egypt	4 (11.1%)

At the time of presentation, *Pseudomonas* was the most frequent organism cultured from respiratory secretions in 8 (22.2%) patients, and details of sputum culture, CT chest finding, and stool analysis are shown in Table 4.

Discussion

In developing countries, CF had remained largely unrecognized. Its clinical features individually resemble those of other diseases such as pneumonia, bronchiectasis, asthma, failure to thrive, and celiac disease. Respiratory and gastrointestinal problems associated with malnutrition and a high infant mortality rate are very common in developing countries, and the diagnosis of CF can therefore be missed due to a low index of suspicion. If clinicians believe that CF is absent from their population, they will not consider it in a differential diagnosis [14].

The present study showed a high frequency of CF (36%) of patients referred to our hospital suspected clinically of having the disease. This high frequency compared to other studies from Egypt probably indicates that the better awareness of CF and the increasing availability of diagnostic tests (the sweat test and/or DNA tests) frequently lead to the identification of a higher number of affected individuals [14]. More than 98% CF

Table 1 Demographic data and clinical presentations of the study population.

Characteristics	All study population N = 100	CF patients N = 36	Sensitivity	Specificity
Age in years (Mean \pm SD)	3.65 \pm 3.87	3.91 \pm 4.18	–	–
<i>Gender</i>				
Male	61 (61%)	22 (61%)	61.11%	39.06%
Female	39 (39%)	14 (39%)	38.89%	60.94%
Consanguinity	53 (53%)	18 (50%)	50%	45.31%
Failure to thrive	56 (56%)	27 (75%)	75%	54.69%
Chronic cough	84 (84%)	32 (88.9%)	88.89%	18.75%
Hemoptysis	6 (6%)	3 (8.3%)	8.33%	95.31%
Nasal Polyps	12 (12%)	4 (11.1%)	11.11%	87.50%
Sinusitis	12 (12%)	3 (8.3%)	8.33%	85.94%
Steatorrhea	37 (37%)	24 (66.7%)	66.67%	79.69%
Hepatobiliary System affection	17 (17%)	5 (13.9%)	13.89%	81.25%
Meconium ileus	2 (2%)	1 (2.8%)	2.78%	98.44%
Clubbing	26 (26%)	11 (30.6%)	30.56%	76.56%

Table 3 Clinical characteristics of CF patients.

Variables (<i>N</i> = 36)	No. (%)
<i>Onset of diagnosis</i>	
During 1st year	12 (33.33%)
From 2–5 years	14 (38.89%)
After 5 years	10 (27.78%)
<i>Onset of symptoms</i>	
Neonatal period	15 (41.67%)
Infancy	16 (44.44%)
From 2–5 years	3 (8.33%)
After 5 years	2 (5.56%)
<i>History</i>	
Chronic cough	32 (88.9%)
Failure to thrive	27 (75%)
Appetite (good)	26 (72.2%)
Steatorrhea	24 (66.7%)
Hepatobiliary system affection	5 (13.9%)
Hemoptysis	3 (8.3%)
Sinuses involvement	3 (8.3%)
Meconium ileus	1 (2.85)
History of recurrent hospital admission	28 (77.8%)
History of ICU admission	22 (61.1%)
<i>General examination</i>	
Ht below 3rd percentile	17 (47.2%)
Wt below 3rd percentile	23 (63.8%)
Pallor	12 (33.3%)
Clubbing	11 (30.5%)
Cyanosis	5 (13.8%)
Nasal polyps	4 (11.1%)
<i>Chest examination</i>	
Diminished air entry	12 (33.3%)
Crepitation	28 (77.7%)
Wheezes	24 (66.6%)
Bronchial breathing	12 (33.3%)
<i>Cardiac examination</i>	
Manifestations of pulmonary hypertension	2 (5.6%)
<i>Abdominal examination</i>	
Abdominal distension	21 (58.3%)
Hepatomegaly	5 (13.8%)

patients have a positive sweat test [15]. So for most patients with a typical clinical picture, the usual problem is not interpreting the result, but remembering to ask for the test.

In Egypt, the first study aiming at evaluating the magnitude of the CF problem in Egypt was done by Abdel Salam and colleagues 1993 using the meconium BM-mec-test and reported a prevalence rate of 1:2664 in 18,560 screened newborns and 1:56 in a series of 224 high risk children [9]. In another more recent study done by Naguib and colleagues, 61 patients suspected of having CF were screened using the CF Indicator sweat test system (PolyChrome Medical, Inc., Brooklyn Center, MN) for qualitative assessment of the sweat chloride concentration. Of the 61 patients, 12 (20%) had positive sweat chloride screening. Ten of the 12 patients underwent quantitative sweat testing and were positive [10].

Some reports have been published about CF patients in the Middle East [9,10] [16–28]. These reports showed the following frequencies, 1:5800 in Bahrain [19], 1:2650 in Jordan, 1:2560 in Kuwait [20], and 1: 15,876 in United Arab Emirates [21].

Table 4 Summary of the laboratory and radiological Investigations done for CF patients.

Investigations	No. (%)
Sweat chloride test results (Mean \pm SD) (<i>n</i> = 36)	86.8 \pm 14.7 mmol/L
<i>Gene analysis for ΔF508</i> (<i>n</i> = 32)	
Negative	10 (27.8%) (0.167 – 0.501) ^b
Heterozygous	14 (38.9%) (0.268 – 0.621) ^b
Homozygous	8 (22.2%) (0.121 – 0.437) ^b
<i>CT chest results</i> (<i>n</i> = 36)	
Normal	4 (11.1%)
Bronchiectasis and hyperinflation	11 (30.6%)
Consolidation	10 (27.8%)
Consolidation collapse and hyperinflation	6 (16.7%)
Consolidation collapse	5 (13.8%)
<i>Stool analysis for fat</i> (<i>n</i> = 36)	
Positive	26 (72.2%)
Negative	10 (27.8%)
<i>Results of Sputum cultures</i> (<i>n</i> = 36)	
<i>Pseudomonas aeruginosa</i>	8 (22.22%)
<i>Klebsiella pneumoniae</i>	2 (5.55%)
<i>Staphylococcus aureus</i>	2 (5.55%)
Mixed flora	2 (5.55%)
Candida	1 (2.77%)
<i>E-coli</i>	1 (2.77%)
<i>Streptococci</i>	1 (2.77%)
No growth	19 (52.82%)

^a Δ F508: deletion of phenylalanine 508 of the cystic fibrosis transmembrane conductance regulator.

^b 95% Confidence intervals for the proportion.

Though CF was generally believed to be rare or nonexistent in Saudi Arabia, in one study, 21 Saudi children were diagnosed as having CF, evidenced by typical clinical features and elevated sweat chloride concentrations from seven referral centers over a period of 10 years [22].

The few studies available about CF in Arabs are suspecting the presence of many undiagnosed patients and emphasize a higher incidence rate particularly in view of the high consanguinity rate in the range of 25–60% [24].

Furthermore, the population of the region is characterized by large family size, high fertility rates, high maternal and paternal age, and high rate of marriage among members of the same tribes. In Bahrain, Al Mahroos found consanguineous marriage in 80% of his study cases [19], and in Lebanon, Desgeorges et al. detected a 50% rate of consanguineous marriage [25]. The present study also revealed a high rate of consanguineous marriage of 53% for the study population and 50% for the CF population which is higher than the reported rate for the general population in Egypt which is 37% [29]; however, the study done by Naguib et al. showed a higher rate of consanguineous marriage reaching to 84% [10].

In the present study, 6 patients (16.7%) gave a positive family history of CF, compared to the study done by Naguib et al. where 23% of CF patients have suggestive family history [10].

In our patients' population, males represent 70% of the whole study population and 86.6% in the CF patients. This finding could reflect a true increase in disease incidence in males or may suggest more severe disease in females with early demise which needs further studies to identify the cause. This

finding may also reflect greater concern and care for male offspring which is deeply rooted in Arab traditions.

CF diagnosis was established in the present study by the age of 1 year in 12 patients (33.33%). In the United States, the majority of cases (71%) were diagnosed by the same age [15]. Shaha and colleagues 2006 diagnosed 58.3% of their patients in the first 6 months of life [30]. It is likely that a delayed diagnosis contributes significantly to the high prevalence of malnutrition in CF patients [25] and could also lead to progressive pulmonary disease and a shortened life span [29].

Due to genetic and environmental differences among ethnic groups, CF presentation may vary between populations. In the present study, 32 (88.9%) patients had chronic or recurrent respiratory disease, 27 (75%) had failure to thrive, and 24 (66.7%) had an abnormal stool pattern "steatorrhea" at the time of diagnosis. These results are similar to those of Al Mahroos, who detected failure to thrive in 96% and progressive lung disease in 84% of Bahraini patients [19]. These results are also close to the study of Shaha et al., with pulmonary problems in 80.6% and failure to thrive in 83.9% of their Pakistani patients [30]. Different reports from the North American CF Registry 2003 indicate that 40.3% of CF patients had failure to thrive, 48.8% had respiratory symptoms and abnormal stools/steatorrhea at time of diagnosis [31]. These 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 (jaundice, hepatomegaly, or cholelithiasis) in CF patients. Hepatobiliary involvement was reported in (4%) to (10.9%) of Middle Eastern patients diagnosed with CF [19,16]. In the study of Naguib et al., there was only one patient with hepatobiliary involvement [10]. In the present study, five of our patients (13.9%) had hepatobiliary manifestations; this frequency of hepatobiliary involvement is much higher than that reported in the North American CF Registry [31].

Analysis of the delta F508 mutations done in CF patients revealed 8 (22.2%) patients with homozygous mutation. This is close to the study done by Naguib et al. in which the delta F508 mutation was detected in 25% of CF patients [10]. This is in contrast to Caucasians where the major mutation F508 deletion is found in about 70% of CF alleles, but all other mutations are rare with a frequency ranging from 2% to less than 0.01% [32].

Fourteen patients (38.9%) with heterozygous mutation were encountered and 10 (27.8%) were negative. Those patients had the classic phenotypic features of CF, so it is suggested that other mutations rather than delta F508 could be the underlying cause. Two known CF disease-producing mutations (e.g., DF508), in the setting of an appropriate disease phenotype, establish the diagnosis of CF; however, failure to find two CF disease-producing mutations cannot exclude the diagnosis of CF. This may be explained by the fact that to date, more than 1800 mutations have been reported, and of these, only around 36 could be tested for by commercially available panels in Egypt. Searching for the remaining mutations requires complete sequencing and referral to specialized labs. In addition, there are mutations that appear to be more widely spread throughout the Middle East but are rarely observed elsewhere. In some cases, these more frequent mutations may be specific for a subset of the people in the Middle East defined by a common ethnic or religious background,

e.g., the 1548delG mutation in Saudi Arabia [18,26], Bedouin tribes in the case of I1234V [27], the S549R (T > G) mutation in Bedouins from the United Arab Emirates and Oman [21], and the 548A > T mutation in Bahrain [28].

Although these studies are diverse and the populations examined were quite small in number, they suggest that the CF problem in the Arab region and Egypt has been underestimated and requires further investigation. To shed light on the actual magnitude of the CF problem in Egypt, further large nationwide screening studies are required.

Conclusions

This study showed a high prevalence of CF among suspected patients, which is more than expected for our population from previous studies. Quantitative Sweat chloride testing is a crucial step in the work up done for patients with clinical suspect of CF. It may be complemented with gene analysis especially in patients with typical clinical picture and equivocal or negative sweat chloride test. The spectrum and distribution of CFTR mutations in Egypt could be defined by screening the complete CFTR gene in CF patients. This will allow a suitable mutation panel to be set up for the Egyptian patients.

Conflict of interest

The authors have declared no conflict of interest.

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