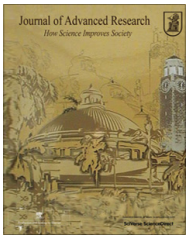
Journal of Advanced Research (2014) 5, 563–568

Cairo University

Journal of Advanced Research



ORIGINAL ARTICLE

Profile of cystic fibrosis in a single referral center in Egypt

Mona M. El-Falaki [a], Walaa A. Shahin [a,*], Noussa R. El-Basha [a], Aliaa A. Ali [a], Dina A. Mehaney [b], Mona M. El-Attar [a]

[a] *Pediatric Department, Faculty of Medicine, Cairo University, Egypt*
[b] *Clinical and Chemical Pathology Department, Faculty of Medicine, Cairo University, Egypt*

ARTICLE INFO

Article history:
Received 11 March 2013
Received in revised form 7 July 2013
Accepted 8 July 2013
Available online 15 July 2013

Keywords:
CF
Children
Sweat chloride
ΔF508 mutation
Egypt

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.

Introduction

CF is the most common potentially lethal and life-shortening genetic diseases among populations of white Caucasian descent, 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].

* Corresponding author. Tel.: +20 1220088310.
E-mail address: walaa_shahin25@hotmail.com (W.A. Shahin).

Peer review under responsibility of Cairo University.

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http://dx.doi.org/10.1016/j.jare.2013.07.005

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 decent.

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, $\geqslant 60$ 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′-ggcaccattaaagaaaatatcatctt-3′, forward mutant-5′-ggcaccattaaagaaaatatcattgg-3′, and common reverse 5′-gttggcatgctttgatgacgcttc-3′. The PCR components were as follows: 10× Buffer without MgCl2, 50 mM MgCl2, 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