Original Article

Cystic fibrosis prevalence among a group of high-risk children in the main referral children hospital in Iran

Mohammad Reza Modaresi, Jamal Faghinia[1], Mohsen Reisi[1], Majid Keivanfar[1], Shiva Navaie, Javad Seyyedi[2], Faride Baharzade[1]

Abstract:

BACKGROUND: Knowledge about cystic fibrosis (CF) in Iran is very limited. The objective of this study was to determine the prevalence of CF among a group of high-risk children with suggestive clinical features in the main referral hospital in Iran.

MATERIALS AND METHODS: This study children consisted of 505 patients who had presented with one or more of the following symptoms: chronic or recurrent respiratory symptoms, gastrointestinal symptoms as rectal prolapse, steatorrhea, hepatobiliary disease as prolonged jaundice, failure to thrive, hyperglycemia and glycosuria, hypochloremic metabolic alkalosis, hypoprothrombinemia, anemia or edema, and positive family history of CF. Patients were screened using pilocarpine iontophoresis to collect sweat and chemical analysis of its chloride content with classic Gibson and Cooke technique.

RESULTS: Of 505 patients, 89 (17.6%) had positive sweat chloride screening test. Five (1%) patients had required cystic fibrosis transmembrane conductive regulator protein mutation analysis to confirm CF.

CONCLUSION: Our findings suggest that in Iran, CF is more common than what previously anticipated. Larger studies are warranted to identify the incidence, molecular basis, and clinical pattern of CF in the Iranian population.

Keywords:

Children, cystic fibrosis, cystic fibrosis transmembrane conductive regulator, Iran

Department of Pediatric Pulmonology, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, [1]Department of Pediatric Pulmonology, Child Growth and Development Research Center, Faculty of Medicine, Research Institute for Primordial Prevention of Noncommunicable Disease, Isfahan University of Medical Sciences, Isfahan, [2]Department of Pediatric Pulmonology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

Address for correspondence:
Dr. Mohsen Reisi, Imam Hossein Hospital, Imam Khomeini Street, Isfahan, Iran.
E-mail: mohsenreisi72@yahoo.com

Introduction

Cystic fibrosis (CF) is a multisystem disorder, requiring diverse knowledge and a complex multifaceted care. CF is the most common autosomal recessive disorder in white people, with a frequency of one in 2000–3000 live births. Most of the morbidity and more than 90% of the mortality of CF are related to chronic pulmonary infection and its complications.[1-6]

CF is caused by a mutation in a gene that encodes cystic fibrosis transmembrane conductive regulator (CFTR) protein, which is expressed in many epithelial and blood cells. CFTR functions mainly as a chloride channel although it has many other regulatory roles; therefore, CFTR mutations would lead to increased viscosity of the mucous secretion that in turn would affect airways, pancreas, liver, and gut, with high morbidity and reduced life expectancy.[1-4]

More than 1500 CFTR mutations have been identified, and the frequency of the mutations varies from population to population worldwide.[7,8]

CF-related symptoms appear throughout life, with great overlap and variability of symptoms and timing from patient

to patient. Life expectancy of the CF patients has dramatically improved in the past 10–20 years, largely as a result of earlier diagnosis, more aggressive therapy, and provision of care in specialized CF center.[8-10]

A number of studies of CF patients in the Middle East have described the clinical presentation and limited genetic analysis of the disease. The studies of Kamal and Nazer in Jordan,[11] Najada *et al*. in Saudi Arabia,[12] and Al-Mahroos in Bahrain[13] revealed that many children with CF in these populations probably remain undiagnosed due to lack of clinical suspicion and proper diagnostic facilities. Naguib *et al*.[14] presented 61 patients who were referred from the chest unit. Patients presented with persistent or recurrent respiratory symptoms, failure to thrive (FTT), diarrhea and/or steatorrhea, and unexplained persistent jaundice. Twelve patients (20%) had positive sweat chloride test. The study results suggested that CF is more common in Egypt than previously anticipated.

CF is increasingly detected in South and East Asia, Africa, and Latin America, with increasing use of newborn screening. However, the incidence and prevalence of CF are still unclear in many areas, in particular in Iran.[15]

As we mentioned, in Iran, limited experience exists about CF. According to the experts' idea, CF maybe more common in Iran than expected before. The objective of this study is to screen for CF in high-risk children with suggestive clinical features.

Materials and Methods

This cross-sectional study was conducted in 2010–2011. Target population consisted of children aged 0–15 years, who were screened at the Children's Medical Center in Tehran, which is the main referral center in Iran.

Patients presented with one or more of the following symptoms were asked to participate in the study:
- Chronic or recurrent respiratory symptoms such as chronic cough, recurrent pneumonia and/or persistent radiographic abnormalities, nasal polyps, and chronic sinuous diseases
- Gastrointestinal symptoms such as chronic diarrhea, steatorrhea, rectal prolapse, meconium ileus, meconium plug syndrome, meconium peritonitis, or hepatobiliary diseases, such as biliary cirrhosis, prolonged jaundice, and FTT
- Hyperglycemia and glycosuria in the second decade of life (without DKA or obesity or type 2 diabetes)
- Hypochloremic metabolic alkalosis
- Hypoprothrombinemia with no other diagnosis explanation
- Anemia or edema with unknown cause
- Positive family history of CF.

This study was approved by the Research and Ethical Committee of Isfahan University of Medical Sciences.

Five hundred and five children were recruited in this study. The questionnaires were filled in; pediatricians examined all patients carefully and referred them for sweat testing and blood sampling for DNA extraction and CFTR gene mutation analysis.

The diagnosis of CF was based on the existence of two elevated sweat chloride concentrations ($Cl \geq 60$ mEq/L) in combination with the presence of the abovementioned typical clinical features or a history of CF in a sibling.[16]

Individuals with intermediate values (30–59 mEq/L for infants under age 6 months and 40–59 mEq/L for older individuals) had undergone repeat sweat chloride testing. If the result of repeated test was Cl - ≥ 60, the diagnosis was confirmed, and if it was in the same ranges (30–59 mEq/L for infants under age 6 months and 40–59 mEq/L for older individuals), CFTR gene mutation analysis was required to confirm diagnosis to be considered in further studies. CF was unlike in infants up to age 6 months with values ≤ 29 mEq/L and in individuals over 6 months with values of ≤ 39 mEq/L.[17]

The classic sweat test, Gibson and Cooke technique, is a standard approach to diagnosis CF and was used in this study. First, the skin of the arm is cleaned using distilled water and wiped up using gauze. Next, 2.5 cm × 2.5 cm copper electrodes are placed on the skin using straps on gauze embedded in pilocarpine nitrate solution (positive electrode) and sulfuric acid 0.004N (negative electrode). A current of 2–5 mA is applied during 5 min. Next, the skin is cleaned again using distilled water and wiped up using gauze for the placement of a filter paper of around 4 cm of diameter covered with plastic and masking tape. After 30–60 min, the paper is removed using tweezers and weighed using an analytical scale to check the sweat mass. Next, the paper is placed inside a glass container, which is sealed with plastic so that it can be sent for sodium and chloride laboratory analysis. Chloride concentration is measured using a digital chloridometer, and the sodium concentration is defined using a flame photometer.[18]

Results

In this study, 505 patients were studied; 280 (55.4%) were male and 225 (44.6%) were female. Eighty-nine (17.6%) patients had positive sweat chloride screening test. CF was confirmed in 46 (16.4%) male and 43 (19.1%) female patients. Four (1.4%) male and one (0.4%) female patients required CFTR gene mutation analysis to confirm CF. There was no gender-specific difference ($P > 0.05$).

The age distribution of participants is shown in Table 1. The mean age of patients with positive and negative CF diagnosis was 40.03 ± 33.3 and 42.9 ± 41.7 months, respectively, without statistical difference between two groups ($P = 0.63$).

As shown in Figure 1, 35 of CF-positive patients and 28 of CF-negative patients had respiratory symptoms, which was not significantly different ($P > 0.05$). Eighty-eight of CF-positive patients and 71 of CF-negative patients had gastric symptoms, which was not significantly different ($P > 0.05$).

The frequency distribution of sign and symptoms is presented in Table 2. According to Table 2, steatorrhea, hepatobiliary disease, FTT, excessive sweating, and positive family history were statistically different between groups ($P < 0.05$).

In 411 (81.4%) patients, the diagnosis of CF was ruled out, and in 89 (17.6%) of patients, the CF diagnosis was confirmed. Seventy-two (14.3%) patients had positive sweat test (sweat Cl >60), fifty patients had intermediate sweat test, and 383 patients had negative sweat test (Cl <29 for age <6 m and Cl <39 for age >6 m). In supplementary checking in patients with intermediate sweat test, 28 cases had negative sweat test and five required genetic assessment.

Discussion

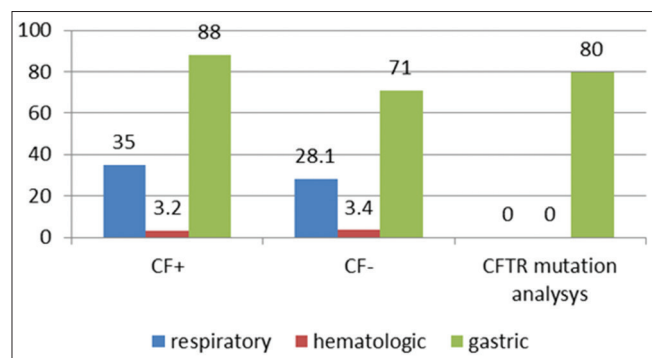CF has been believed to occur infrequently in Iran. Karjoo *et al*. performed pilocarpine iontophoresis on 125 suspected patients and twenty controls from Fars Province in Southern Iran. Only three patients were found to have CF on the basis of the clinical findings, repeated elevations of sweat chloride levels, and evidence of pancreatic insufficiency,[19] but the present study suggests that the occurrence of CF in Iran is higher in high-risk children than previously thought. In our study, CF was diagnosed in 17.6% of high-risk children. Few reports have been published about CF patients in the Middle East.[18-20] These reports indicated frequencies ranging from 1:5800 in Bahrain[13] to 1:2650 in Jordan.[21] Naguib *et al*.[14] studied 61 patients who presented with persistent or recurrent respiratory symptoms, FTT, diarrhea and/or steatorrhea, and unexplained persistent jaundice. Twelve patients (20%) had positive sweat chloride test. The prevalence of CF in the European Union[22] has been reported 0.737/10,000 in the 27 EU countries, which is similar to the value of 0.797 in the United States.

The lack of definitive data about the prevalence of CF in Iran may cause misdiagnosis of this disease, where a significant proportion of infant morbidity and mortality is caused by respiratory infections, diarrheal disease, and malnutrition.[10,23] 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 Iran regarding the presentation and diagnosis of the disease might be a contributing factor to underdiagnosis of the disease.

The clinical presentations of CF are variable. Due to the genetic and environmental differences among ethnic groups, CF presentation may also vary between populations.[25] In the present study, 27 (30.3%) of CF-positive patients had FTT at the time of diagnosis, 35% of CF patients had chronic respiratory disease, and 18 (20.2%) patients had steatorrhea. In contrast, reports from the North American CF Registry[24,25] indicate that 40.3% of CF patients had FTT, 48.8% had respiratory symptoms, and 32.2% had abnormal stools/steatorrhea at the time of diagnosis. In a small study in Egypt,[14] all CF patients had FTT, 92% had chronic or recurrent respiratory disease, and 58% had abnormal stools. The present study did select high-risk patients with chronic illness; therefore, findings may be influenced by a

Figure 1: Frequency of sign and symptoms in the three groups studied

Table 1: Age distribution of study participants

Age	Frequency, *n* (%)	CF positive, *n* (%)	CF negative, *n* (%)	CFTR mutation analysis requirement, *n* (%)
0-6 months	68 (13.5)	13 (19.1)	54 (74.9)	1 (1.5)
6-12 months	76 (15)	7 (9.2)	69 (90.8)	0
1-2 years	113 (22.4)	25 (22.1)	88 (77.9)	0
2-5 years	147 (29.1)	30 (20.4)	114 (77.4)	3 (2)
5-15 years	101 (20)	14 (13.9)	86 (85.1)	1 (1.5)
Total	505 (100)	89 (17.6)	411 (81.4)	5 (1)

CF = Cystic fibrosis, CFTR = Cystic fibrosis transmembrane conductive regulator

Table 2: Frequency of signs and symptoms in patients with sweat positive and negative test

Signs and symptoms	Levels	CF negative, *n* (%)	CF positive, *n* (%)	CFTR gene analysis, *n* (%)	P
Chronic cough	Yes	34 (6.7)	8 (9)	0	0.55
	No	377 (93.3)	81 (91)	5 (100)	
Recurrent pneumonia	Yes	29 (7)	7 (7.9)	0	0.47
	No	382 (93)	82 (92.1)	5 (100)	
Nasal polyp	Yes	6 (1.46)	1 (1.1)	0	1
	No	405 (98.54)	88 (98.9)	5 (100)	
Chronic sinusitis	Yes	9 (2.2)	1 (1.1)	0	1
	No	402 (98.8)	88 (98.9)	5 (100)	
Other chronic respiratory symptoms	Yes	66 (16)	8 (9)	0	0.35
	No	345 (84)	81 (91)	5 (100)	
Diarrhea	Yes	63 (12.3)	9 (10.1)	0	0.74
	No	348 (87.7.)	80 (89.9)	5 (100)	
Steatorrhea	Yes	34 (8.3)	18 (20.2)	0	<0.001
	No	377 (91.7)	71 (79.8)	5 (100)	
Rectal prolaps	Yes	6 (1.5)	1 (1.1.)	0	1
	No	405 (98.5)	88 (98.9)	5 (100)	
Hepatobiliary and prolonged icterus	Yes	50 (12.2)	2 (2.2)	0	0.02
	No	361 (87.8)	88 (97.8)	5 (100)	
FTT	Yes	178 (43.3)	27 (30.3)	4 (80)	0.049
	No	233 (56.7)	62 (69.7)	1 (20)	
Other gastrointestinal symptoms	Yes	31 (7.5)	6 (6.7)	0	0.86
	No	380 (92.5)	83 (93.3)	5 (100)	
Hyperglycemia	Yes	2 (0.5)	0 (0)	0	1
	No	409 (99.5)	89 (100)	5 (100)	
Anemia	Yes	4 (1)	1 (1.1)	0	0.56
	No	407 (99)	88 (98.9)	5 (100)	
Edema	Yes	9 (2.2)	2 (2.2)	0	0.69
	No	402 (97.8)	87 (97.8)	5 (100)	
Positive family history	Yes	8 (1.9)	5 (5.6)	1 (20)	0.012
	No	403 (98.1)	84 (94.4)	4 (80)	
Sweat	Yes	21 (5.1)	10 (11.2)	0	0.016
	No	390 (94.9)	79 (88.8)	5 (100)	

FTT = Failure to thrive, CF = Cystic fibrosis, CFTR = Cystic fibrosis transmembrane conductive regulator

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%[23-26]–10.9%[21,27] of Middle Eastern patients diagnosed with CF. In this study, hepatobiliary diseases occurred in fifty patients, of which 2 (2.2%) had positive sweat test. However, the studies are diverse and the findings may represent a different spectrum of CF manifestations in the Middle East.

In our study, 81 (91%) of positive cases have been diagnosed before 7 years of age. One of the positive subjects was a 14-year-old female child who was referred due to chronic sinusitis.

11.2% of positive cases had excessive sweating and it might be important to consider CF in the differential diagnosis of children who are referred to the clinics by sweating and other diagnoses have been ruled out.

Conclusion

This study reveals the need for further investigation of CF in Iranian children at an early age. Previously thought to be extremely rare, the true incidence of the disease in Iran needs to be established. Its true frequency may be obscured by more common conditions including respiratory infections, diarrhea, and malnutrition, as well as other genetic or metabolic diseases. Further analysis of the clinical manifestations of the disease in Iranian children and characterization of the mutation spectrum are therefore warranted. Finally, establishment of a CF center to provide specific treatment plans is necessary to improve the quality of life and to prolonged patient survival. As part of this effort, the molecular basis of CF should be characterized in Iranian patients to identify common mutations in this population and to provide confirmation of the clinical diagnosis.

and Children Growth Research Center with the approved code number: 408125.

Conflicts of interest

There are no conflicts of interest.

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