

SCREENING FOR CYSTIC FIBROSIS: THE IMPORTANCE OF USING THE CORRECT TOOLS

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Background: Cystic Fibrosis (CF) is a potentially lethal genetic disorder. The most frequent mutation worldwide in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene is designated as the Delta F508 mutation. This mutation was found in only 33% of Pakistani patients studied. Since the common Pakistani mutations remain to be identified, appropriate screening tools are required to identify disease. Sweat chloride determinations remain the gold standard for diagnosing CF. This study was done to emphasize the importance of using the correct tests. **Methods:** The study was conducted at the Aga Khan University Hospital. The *CFTR* delta F508 mutation was tested on blood samples from patients suspected with CF. Sweat chloride analysis using pilocarpine iontophoresis was done with a positive value of greater than 60 meq/L. **Results:** 57 pediatric samples were screened for the delta F508 mutation and were positive in only 10.6 % of all patients tested. 12/57 (21%) had a preliminary sweat test. 6/12 (50%) of these patients had an abnormal sweat test and 3/6 patients with an abnormal sweat chloride (50 %) had deltaF508 mutations - 2/6 (33%) were homozygotes and 1 was a compound heterozygote. Since 79% did not have a sweat test, it was difficult to assess whether this subset of patients had cystic fibrosis with a *CFTR* mutation other than the delta F508 tested or no CF. **Conclusion:** Sweat chloride analysis is critical to distinguish CF from other causes of severe pulmonary and pancreatic insufficiencies and to define patients requiring further analysis. **Key words:** Cystic fibrosis, sweat chloride, mutation analysis

INTRODUCTION

Cystic Fibrosis is the most common autosomal recessive, potentially lethal genetic disorder, associated with pulmonary and pancreatic insufficiency. The abnormality lies in a mutation in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene. More than 1340 mutations in this gene have been identified worldwide to date. The most frequent mutation identified is designated as the Delta F508 mutation. This mutation, which is present in 70-100 % of CF patients of Caucasian origin, was found in only 33% of the Pakistani patients that we have studied so far (1). The CF mutations in the remaining 66 % of Pakistani patients have yet to be defined.

Screening tools, such as sweat chloride analysis, are critical for the identification of those patients that would require further mutation analysis. Since the common Pakistani mutations still remain to be identified, sweat chloride determinations are extremely important to make a diagnosis of Cystic fibrosis. This study was done to emphasize the importance of using the correct screening tools to establish an unambiguous diagnosis of the disease.

MATERIAL AND METHODS

The study was conducted at the Aga Khan University Hospital, Karachi, Pakistan over the years 2002-2003. This is a tertiary care facility that caters to

patients from all over Karachi as well as from other areas of Pakistan.

Patients with clinical suspicion for CF were identified. The mutation analysis for *CFTR* delta F508 was done on blood samples from all these patients using the amplification refractory mutation system and polymerase chain reactions. The delta F508 test was used as the sole means of diagnosis of CF for a period of time during which the sweat test was unavailable due to malfunction of the machine. The sweat chloride analysis as a screening tool was therefore used in only some of these patients. The sweat test used pilocarpine iontophoresis and a positive value was defined as a sweat chloride of greater than 60 meq/L. Meconium ileus was also considered as a positive outcome variable of CF and these patients were included in the study. We reviewed the charts of all patients hence screened for CF.

RESULTS

The Molecular Biology department at the Aga Khan University received 57 pediatric samples to be screened for the delta F508 mutation. 61.4% of the patients tested were suspected to have CF due to respiratory symptoms, 19.3% for malabsorption and diarrhea and 8.8 % for problems with failure to thrive. 3 patients had meconium ileus as a presenting feature (Table 1). Of all these patients, however, only 12/57 (21%) patients had a preliminary sweat

chloride analysis. 6/12 (50%) of those tested with the sweat chloride analysis had an abnormal sweat chloride value (71-168, mean 109 meq/L) and 3 of these 6 patients (50 %) with an abnormal sweat chloride had delta F508 mutations - 2/6 (33%) were homozygotes and 1 was a compound heterozygote (the other mutation is still unknown). Two of the three patients (66.7%) with meconium ileus had a homozygous delta F508 mutation. (**Table 2**) Overall, the mutation analysis for delta F508 was positive in only 10.6 % of all the patients tested. Importantly since 79% did not have an initial sweat chloride analysis, it would be difficult to assess whether this subset of patients had cystic fibrosis with a *CFTR* mutation other than the delta F508 or no cystic fibrosis at all. Two of the 57 patients (3.5%) did not have a screening sweat test but were heterozygous for delta F508. The sensitivity of the delta F508 test to diagnose CF when used alone is 43% with a specificity of a 100%.

When combined with an initial sweat chloride, the delta F508 mutation analysis was positive in 50% of those with an abnormal sweat test result. We suspect that the remaining 50% may have a *CFTR* mutation other than the delta F508 tested.

Table 1: Presenting features of patients tested for Cystic fibrosis

Clinical features	Percentage of patients (n)
Respiratory symptoms	61.4% (35)
Malabsorption and diarrhea	19.3% (11)
Failure to thrive	8.8% (5)
Liver disease, pancreatic disease and meconium ileus	10.5% (6)

Table 2: Results of sweat chloride and mutation analysis

Miscellaneous features	Number of patients
Patients tested for delta F508	57
Sweat test done	12/57 (21%)
Elevated sweat Chloride	6/12 (50%)
Elevated sweat Chloride with delta F508 allele	3/6 (50%)
Delta F508 with positive sweat chloride	2/6(33%)
Delta F508 in those with no preliminary sweat test	2/45 (4.4%)
Patients with meconium ileus homozygous for delta F508	2/3 66.7%

DISCUSSION

Cystic fibrosis is a disease with varied clinical manifestations. Quinton and Knowles et al² had first suggested that the primary defect of cystic fibrosis

may be in chloride transport. The clinical features of CF stem from the resultant mucous plugging in various organ systems. Allan et al.³ demonstrated that meconium ileus was also a feature of cystic fibrosis. An extension of this is the distal intestinal obstruction syndrome or DIOS known as a 'meconium ileus equivalent' that occurs in adolescents and adults with CF. This obstruction is the consequence of abnormally viscid mucofeaculant material in the terminal ileum and colon of these patients.

Pancreatic Insufficiency is an important feature of cystic fibrosis. However, approximately 15% of CF patients do not have pancreatic insufficiency, i.e., they are 'pancreatic sufficient'. Kerem et al.⁴ have showed that phenotype/genotype correlations dictate whether pancreatic insufficiency or sufficiency is associated with different mutations at the CF locus.

Mucus plugging in the lungs leads to areas of atelectasis and superimposed infections. Patients with cystic fibrosis therefore have an increased predisposition to the development of pulmonary disease. The organisms most frequently cultured from tracheal aspirates from patients with CF include *Pseudomonas* and *Staphylococcus*. Pier et al.⁵ found that cultured human airway epithelial cells expressing the delta-F508 allele of the *CFTR* gene demonstrate defective clearance of *Pseudomonas* that possibly leads to enhanced pulmonary infection with this organism in CF.

Focal and multilobular biliary cirrhosis are considered path gnomonic of liver disease in CF⁶. The prevalence rates and the risk factors for the development of CF liver disease have varied considerably.⁷ It is suggested that gene modifiers may influence disease expression and Gabolde et al have⁸ demonstrated that the presence of cirrhosis in patients with cystic fibrosis may be associated with either homozygous or compound heterozygous mutant mannose-binding lectins.

Congenital bilateral absence of the vas deferens as a cause of male infertility has also been described in cystic fibrosis.

SCREENING

Several guidelines have been prepared worldwide concerning screening for the cystic fibrosis gene⁹. Screening tools considered have included the immune reactive trypsin test and the sweat chloride analysis.

Newborn babies with CF have abnormally high levels of immunoreactive trypsin (IRT) in serum, which has been the basis for its use as a screening tool. The IRT test has a 90% detection rate for CF and a 0.3% false positive rate.

Other tests that have been used for screening for cystic fibrosis include measurement of nasal potential difference and conductance through mucosal biopsies when placed in Ussing chambers.

The only screening tool currently available in Pakistan is the sweat chloride analysis. The Pilocarpine Sweat Test remains the gold standard for diagnosis. This test measures the concentration of chloride in the sweat. In the majority of CF patients with typical features and identified mutations the sweat test is diagnostic. A positive sweat test is then followed by a mutation analysis for the predominant mutations in the population.

The indications for sweat testing include clinical features suggestive of CF, a family history of CF, a positive newborn screening test (such as IRT) and the suspicion of an atypical phenotype. A positive result is described as a sweat chloride of greater than 60 mmol/L, equivocal values are those between 40-60 mmol/L and a negative test is described as values less than 40 mmol/L. A value of 40-60 mmol/L in infancy is however considered suspect for CF^{9,10}. Borderline sweat results are seen with some mutations such as in R117H, R334W, and P67L^{11,12}. Patients with the F508/S1235R may have negative sweat tests.

The sweat test can be done in term infants older than 7 days of age but preferably should be done in infants after 2 weeks of age and when they are greater than 3 kg in weight^{13,14}. In a recent population based study in infants, of the 98 CF-screened-positive affected infants for whom sweat results had been reported, 84/98 (86%) had values that were clearly positive and 13 of the remaining 14 had sweat-test results that are consistent with expectations for a particular CF genotype¹⁵.

The diagnosis of CF can however remain uncertain in patients with clinical features of CF but intermediate sweat chloride values and the absence of identified mutations. It is also important to note that the sweat chloride concentration does not correlate with disease severity and a lower concentration does not necessarily predict a milder pulmonary course in patients with cystic fibrosis^{16,17}.

A positive sweat chloride value is an important tool to distinguish CF from other causes of severe pulmonary and pancreatic insufficiencies. It also serves to define those patients who require further mutation analysis. In the New England Newborn Screening program, 50% of the patients were identified by Delta F508 testing alone while 75% were identified when the mutation analysis was expanded to include several genetic mutations¹⁸. Since, 79% of our patients tested for delta F508, with symptoms suggestive of CF, did not have a preliminary sweat test it was very difficult to assess

whether this subset of patients had CF with a CFTR mutation other than delta F508 or they had a disease other than cystic fibrosis. When combined with an initial sweat chloride, the delta F508 mutation analysis was positive in 50% of those with an abnormal sweat test result. We suspect that the remaining 50% may have a CFTR mutation other than the delta F508 tested. We are now in the process of studying the common mutations in CF in our population.

Only a small fraction 3.5% of the 57 patients in this study had a positive mutation analysis in the absence of a preliminary screening sweat chloride.

SUMMARY

Since delta F508 is not present in 100% of Pakistani CF patients, making the diagnosis of CF on the basis of the presence of one mutation alone would therefore lead to many false negative diagnoses. We are in the process of identifying the common mutations in our population that will help in the establishment of a diagnosis of CF. In the absence of other screening tests in Pakistan, sweat chloride analysis therefore serves as an integral screening tool for CF.

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