Manuscript type: Original Article

DOI: 10.5152/TurkThoracJ.2019.18187

Title: Newborn Screening for Cystic Fibrosis in Mersin Province: Yearly Assessment of the National Program

Short Title: Newborn Screening for Cystic Fibrosis

Authors: Ali Özdemir[1], Dilek Doğruel[2]

Instutions: [1]Pediatric Pulmonary Section, Department of Pediatrics, Mersin City Training & Research Hospital, Mersin
[2]Pediatric Allergy Section, Department of Pediatrics, University of Çukurova, Adana

Address for correspondence: Ali Özdemir

E-mail: aliozdemir@hotmail.com

Received: 26.11.2018
Accepted: 05.03.2019

Cite this article as: Özdemir A, Doğruel D. Newborn Screening for Cystic Fibrosis in Mersin Province: Yearly Assessment of the National Program. Turk Thorac J 2019; DOI: 10.5152/TurkThoracJ.2019.18187

Abstract

Objective: A national newborn screening program for cystic fibrosis (CF) has started by using immunoreactive trypsinogen (IRT) test on January 1, 2015 in Turkey. We aimed to analyze the characteristics of newborn screen positive (NBSP) infants in Mersin province.

Materials and Methods: The data of NBSP infants were retrospectively analyzed between years 2015 and 2017 from records of Hospital and Hospital.

Results: A total of 82,273 newborns were screened for CF by IRT test between years January 2015 and December 2017 in Mersin. Among those, 512 infants were defined as NBSP after two IRT tests (138 infants in 2015, 217 infants in 2016 and 157 infants in 2017, respectively). Sweat test was normal in the majority of infants [115 infants (83.3%) in 2015, 189 infants (87.1%) in 2016, and 129 infants (82.2%) in 2017]. Overall, after two repeated sweat tests, 4 infants had sweat test results in the intermediate range and 9 infants had positive sweat tests between years 2015 and 2017. The

incidence of CF for a 3-year period was approximately 1/9300 in our region. The positive predictive value of IRT test for defining CF was 1.8% with a sensitivity of 90.0% and specificity of 99.4%.

Conclusion: IRT/IRT test as a newborn screening strategy provides the opportunity for earlier diagnosis and treatment of CF patients. More data are needed to understand the frequency of CF on a national base.

Key words: cystic fibrosis, newborn screening, IRT test

INTRODUCTION

Cystic fibrosis (CF) is an autosomal resessive disorder of exocrine gland function mostly affecting lungs, gastrointestinal tract, biliary and liver, sinuses and reproductive system [1]. The lung is the mainly affected organ in CF patients at a rate of 80-85% and recurrent lung infections result in an obstructive chronic lung disease which determines the morbidity and mortality [2]. The disease is common among Northern European descent at a rate of 1/2500 [3]. The incidence of CF in Turkey is unknown. However, there are few studies estimating the rate approximately 1/3000 [4]. Notably, one can assume that the rate is much higher in which consanguineous marriage is common in Turkey.

CF is caused by a genetic mutation defined in 1989 in a gene on chromosome 7q31 that codes for a 1480 aminoacidic protein "transmembrane conductance regulator" (CFTR) protein which functions as a transmembrane cAMP-activated chloride channel [5]. There are over 2000 different mutations in the CFTR gene that can cause disease [6]. The most common mutation is delta F508 (delF508) which is found in 80-85% of North American Caucasian and European CF patients. The mutation in delF508 is reported 23.94% of CF patients in Turkey [7].

The newborn screening test for early diagnosis of CF disease was initially reported in the late 1970's with the measurement of IRT levels and gradually established mainly in North America, Oceanesian countries and Europe with various screening programs [8-10]. The main goal of newborn screening programs is to determine infants with CF at an earlier stage, appropriate follow up in CF specialized care centers, delay or prevent health problems related to CF. A retrospective study from Italy investigating the survival of infants diagnosed with NBSP or through CF-related symptoms between years 1971 and 2014 had shown 30 year survival was better in the NBSP group (80.1%) than CF-related symptom group (71.0%). Additionally, if the data was segregated for severe and moderate

groups, the 20-year survival was significantly higher in the NBSP versus CF-related symptom group in the severe (85% versus 64%) and moderate (94% versus 86%) groups [11].

Turkey has started a national newborn screening program using two repeated immunoreactive tripsinogen (IRT/IRT) test for early diagnosis of infants with CF at the beginning of 2015 [12]. Trypsinogen is an inactive precursor produced by the pancreas that is converted to the enzyme trypsin [13]. Newborns with CF may have elevated blood levels of IRT due to destroyed pancreatic aciner cells. As a newborn screening policy for CF in Turkey, first IRT test is obtained in the first days of life on a dried blood spot specimen, and a cut-off value over 90 µg/l is considered to be positive. Then, a second IRT test is obtained in first IRT positive newborns at the age of 7-14 days. At a level above 70 µg/l, newborns are referred to a specialized center for further investigation and sweat test procedure.

Our pediatric pulmonology center is the only affiliated hospital for referals of newborn screen positive (NBSP) babies in Mersin province since the implementation of nationwide IRT/IRT program starting on January 2015. The initial referal center was Hospital between years January 2015 and February 2016. Then, after clousure of this hospital, Research & Training Hospital has been the center of NBSP referals. The aim of this study was to evaluate the performance of newborn screening program for CF in Mersin province based on the results of first three years.

MATERIALS AND METHODS

The records of Hospital and Research & Training Hospital of all NBSP babies were retrospectively investigated between years 2015 and 2017. The study parameters included birth rate/year in Mersin province, screened newborn population in a year, number of NBSP babies, number of referals from family physicians, infant age at clinic visit, weight, sex and sweat test results. The population and birth rate statistics in Mersin province were obtained from Turkish National Statistics Association database.

Sweat test procedure

Sweat was conducted by an experienced nurse to infants' forearm using pilocarpine iontophoresis. A sweat collection device was attached to one arm and sweat was collected a minimum amount of ≥ 75 mgr for 30 min, and then analyzed for chloride ion concentration (SWEAT ANALYSIS UNIT UCF® 2011). In the presence of insufficient sweat collection, families were asked for a repeated test within a month.

Sweat chloride level < 30 mmol/L was considered normal; 30-59 mmol/L was defined intermediate; ≥ 60 mmol/L was positive result for the diagnosis of CF in infants < 6 months of age. Sweat chloride

level < 40 mmol/L was considered normal; 40-59 mmol/L was defined intermediate; ≥ 60 mmol/L was positive result for the diagnosis of CF in infants ≥ 6 months of age. If the sweat chloride level was in the intermediate or positive range, a second test was recommended to confirm the diagnosis of CF or CFTR related metabolic syndrome (CRMS) or cystic fibrosis screen positive inconclusive diagnosis (CFSPID).

The study was approved by the ethical committee of the University (Approval No 2018/36).

Statistical analysis

Statistical analysis was performed using the SPSS version 17.0 software statistical package program (SPSS Inc., Chicago, IL). Continuous variables were expressed as mean and SD for parametric data and range for nonparametric data. Categorical variables were expressed as frequency or percentage. Incidence was defined as the number of new cases of a disease within a time period, as a proportion of the number of people at risk for the disease. Positive predictive value was the probability that subjects with a positive screening test truly had the disease. Sensitivity was the probability that a test would indicate disease among those with the disease. Specificity was the fraction of those without disease who would have a negative test result.

RESULTS

A total of 27,969 newborn (screen coverage: 98.86% of born babies) in the year 2015, 27,030 newborn (screen coverage: 96.04% of born babies) in the year 2016, and 27,274 newborn (screen coverage: 99.02% of born babies) were screened by IRT/IRT test. There were 138 NBSP babies in year 2015, 217 NBSP babies in year 2016 and 157 NBSP babies in year 2017 referred to our center for further evaluation. The weight and sex distribution of the NBSP babies were as follow: 3198 ± 565 grams (range: 790-4200 grams), male gender: 65 (47.1%) in year 2015; 3202 ± 547 grams (range: 1600-4900 grams), male gender: 108 (49.8%) in year 2016; 3208 ± 630 grams (range: 660-4910 grams) in year 2017, male gender: 76 (48.4%). (Table 1)

There were some NBSP babies whom did not undergo further evaluation for various reasons (e.g., parental refusal, unreachable address, immigrant family or migration to another area). Seven babies (5.0%) in year 2015, 17 babies (7.8%) in year 2016 and 17 babies (10.8%) in year 2017 were not evaluated for CF in this respect.

The time of sweat testing for NBSP infants referred to our center was 91.8 ± 30.9 days (range: 15-214 days) in year 2015, 53.8 ± 31.2 days (range: 16-207 days) in year 2016, and 119.9 ± 39.4 days (range:

18-201 days) in year 2017. Sweat test result was normal in 115 (%83.3) infants in year 2015, 189 (87.1%) infants in year 2016, and 129 (82.2%) infants in year 2017. Sweat test result in the intermediate range was reported in 13 (9.4%) infants for year 2015, 8 (3.0%) infants for year 2016, and 8 (5.1%) infants for year 2017. The intermediate sweat test reported infants had a second sweat test at the age of 6 months. Among 29 infants, only 4 of them had consistent intermediate sweat test result and considered CRMS/CFSPID. Sweat test result was positive 3 (2.2%) infants in year 2015, 3 (1.1%) infants in year 2016, and 3 (1.9%) in year 2017. These infants also had a second sweat test to confirm the diagnosis of CF. Also, important to note that there was one infant with negative newborn screening test later diagnosed as CF at the age of 7 months in 2016 in our region.

The incidence of CF among NBSP infants was 1/9430 in 2015, 1/9347 in 2016 and 1/9182 in 2017, respectively. The overall incidence of the disease in Mersin was 1/9388 live births.(including newborn screen negative infant with CF). The positive predictive value of IRT test to determine infants with CF was 1.8%. Sensitivity and specificity of the CF newborn screening program were 90.0% and 99.4%, respectively.

Among 9 infants with CF, only 2 were investigated for genetic analysis in another institution (due to lack of genetic laboratory in our center). One infant was homozygous for 2789+5G>A mutation and the other was heterozygous for delF508+ c1520-1522 delTCT mutation.

DISCUSSION

The decision to include newborn screening for CF as a nationwide in Turkey has started by January 2015. Since then, our pediatric pulmonology section has been the referral center of NBSP babies. A total of 82,273 were screened by IRT/IRT method in our region between years 2015 and 2017. Our results showed that the newborn screening for CF in Mersin was highly successful. Hovewer, approximately 8% of NBSP infants did not undergo further evaluation and sweat testing for CF disease. It should be noted that there was an increasing trend to unachievable infants by years. This accessibility rate appears to be one of the most important limitation of newborn screening program in our region. There were 512 newborns defined as NBSP. Among them, 9 infants had the diagnosis of CF and have been currently followed by our clinic. It is important to test infants with CF for genetic analysis. However, it was not possible to reach such an analysis due to lack of genetic laboratory in our hospital and most infants did not have a genetic confirmation. Additionally, there were also 4 infants considered as CRMS/CFSPID by sweat test results. In some cases the sweat chloride result may be intermediate or CFTR gene changes may be recognised which the phenotypic consequences is unclear. The US centers proposed a term for designation of these infants, CFTR-related metabolic

syndrome, and subsequently European guidelines used the term cystic fibrosis screen positive inconclusive diagnosis in the same manner [14,15]. These infants do not have a disease but have a number of risk factors for developing CF related issues in the future. Management of infants with an inconclusive diagnosis after NBSP for CF is challenging. Therefore, the families of such infants should be appropriately informed and followed by care givers.

In general, the incidence of CF implemented by national neonatal screening program is surprisingly lower than expected in our province. It appears to be less regardless of the unscreened or unachievable infants. Such an information should be clarified by other regional or national data when available. It would be ideal if the prevalence of CF could be estimated in our region. In order to estimate such a parameter, total number (i.e. previously and newly diagnosed) of CF patients should be known in the region. Unfortunately, the real number of all CF patients in our province is not well-known. Positive and negative predictive values are also influenced by the prevalence *o*f disease in the population that is being tested. Therefore, an approximate positive predictive value of IRT test was given in our results.

There are several studies that investigated the performance of IRT test as a newborn screening strategy for CF [16-18]. In a regional study conducted in São Paulo State, Brazil using IRT/IRT protocol, the incidence of CF was reported 1/8403 among 60,000 screened newborns. The rate of false positive result was 95.2% and the positive predictive value for the IRT test was 8% [19]. A 10-year analysis of reviewing the effectiveness of IRT testing in Victoria state, Australia had proven succesful in detecting most babies with CF. The incidence was given 1/2874. However, 9 cases of CF out of 191cases were missed by IRT test [20].

Today, different methodologies are used to screen for CF in newborns among countries. In all programs, the first stage of screening entails measurement of IRT on dried blood spots. Then, the second stage involves a repeat IRT (IRT/IRT), DNA test for common CF mutations (IRT/DNA) or pancreatic associate protein (IRT/PAP) [21]. Newborn screening using IRT/DNA method should have commonly known mutations for CF in a targeted population. The recommended panel of mutations must account for > 80% of CF alleles. A retrospective review from the US evaluating the performance of IRT/IRT and IRT/DNA methodology as a newborn screening for CF had shown CFTR-DNA panel sensitivity was 96.2%, compared with sensitivity of 76.1% observed with IRT/IRT (at 105 ng/mL cut-offs) [22]. An alternative newborn screening methodology developed in Europe that uses the IRT/PAP test has also shown compatible sensitivity and specificity in the diagnosis of infants with CF [23].

There are advantages and disadvantages for each screening programs. A screening test is not meant

to be diagnostic and therefore will always have some “false positives”, regardless of methodology. Therefore, all children who screen positive should undergo a diagnostic sweat test.

Importantly, The Turkish National CF Registry has been established at the beginning of year 2018 and most CF centers are started recording their data to the system. The CF Registry is a secure centralised database, sponsored and managed by the University of Hacettepe. Registry data will be used to improve the health of people with CF through research, to guide quality improvement at care centers. The National CF Registry is also integrated to the European CF Society Patient Registry which annually reports the demographic and clinical data from people with CF throughout Europe and neighbouring countries. Our center is initially joined to the national program and contribute the data of CF patients to the system from Mersin province.

To our knowledge, there has been no published data regarding the established newborn screening program for CF in our country other than reports presented in National Congresses [24]. Therefore, it is important to document national and regional data for the newly established newborn screening program. First, it will provide the prevalence and incidence of CF in Turkey. Second, documentation of data will let us understand success or failure parts of the ongoing screening program. And last, CF patients identified by screening will be followed by specialized CF centers, and many of CF associated problems with a late diagnosis can be prevented by early diagnosis and such an approach improve the quality of life in CF patients.

In conclusion, the performance of newborn screening program using IRT test for CF in Mersin province appears to be highly succesful. The program has allowed early diagnosis of CF patients in our region.

Since the study was retrospective, no parental consent was obtained.

REFERENCES

1.Anselmo MA, Lands LC. Cystic fibrosis: overview. Taussig LM, Landau LIL, Le Souef PN, Morgan WJ, Martinez FD, Sly PD, editors. Pediatric Respiratory Medicine (2nd ed). Philadelphia: Mosby; 2008. p. 845-57.

2.Tiddens H, Rosenfeld M. Cystic fibrosis: respiratory manifestations. Taussig LM, Landau LIL, Le Souef PN, Morgan WJ, Martinez FD, Sly PD, editors. Pediatric Respiratory Medicine (2nd ed). Philadelphia: Mosby; 2008. p. 871-87.

3.Zvereff VV, Faruki H, Edwards M, et al. Cystic fibrosis carrier screening in a North American population. Genet Med 2014;16:539-46.

4. Kiper N. Kistik Fibrozis; Ülkemizdeki Durum. J Curr Pediatr 2007: 5.

5. Collins FS, Riordan JR, Tsui LC. The Cystic Fibrosis Gene: Isolation and Significance. Hosp Pract 1990 15; 25:47-57.

6. Sosnay PR, Raraigh KS, Gibson RL. Molecular Genetics of Cystic Fibrosis Transmembrane Conductance Regulator: Genotype and Phenotype. Pediatr Clin North Am 2016;63:585-98.

7. Available from https://www.ecfs.eu/projects/ecfs-patient-registry/annual-reports.

8. Crossley JR, Elliott RB, Smith PA. Dried-blood spot screening for cystic fibrosis in the newborn. Lancet 1979;1:472-74.

9. Southern KW, Munck A, Pollitt R, et al. A survey of newborn screening for cystic fibrosis in Europe. J Cyst Fibros 2007; 6:57-65.

10. Borowitz D, Robinson KA, Rosenfeld M, et al. Cystic Fibrosis Foundation evidence-based guidelines for management of infants with cystic fibrosis. J Pediatr 2009; 155: S73–S93.

11.Tridello G, Castellani C, Meneghelli I, et al. Early diagnosis from newborn screening maximises survival in severe cystic fibrosis. ERJ Open Res 2018; 20: pii 00109-2017.

12. Ersu R, Çakır E. Kistik Fibrozis Yenidoğan Tarama Testi ile Tanı Alan Hastaları İzleme Rehberi. Ankara: T.C. Sağlık Bakanlığı Türkiye Halk Sağlığı Kurumu; 2015:6.

13. Li L, Zhou Y, Bell CJ, Earley MC, et al. Development and characterization of dried blood spot materials for the measurement of immunoreactive trypsinogen. J Med Screen 2006;13:79-84.

14. Machogu E, Ren CL. Novel insights into the diagnostic and therapeutic challenges of the CFTR metabolic syndrome/CF screen positive indeterminate diagnosis. Pediatr Pulmonol 2016; 51:S45-S48

15. Barben J1, Southern KW. Cystic fibrosis screen positive, inconclusive diagnosis. Curr Opin Pulm Med 2016; 22:617-22.

16. Southern KW, Munck A, Pollitt R, et al. A survey of newborn screening for cystic fibrosis in Europe. J Cyst Fibros 2007;6:57-65.

17. Calvin J, Hogg SL, McShane D, et al. Thirty-years of screening for cystic fibrosis in East Anglia. Arch Dis Child 2012;97:1043-7.

18. Sands D, Zybert K, Mierzejewska E, et al. Diagnosing cystic fibrosis in newborn screening in Poland - 15 years of experience. Dev Period Med 2015;19:16-24.

19. Rodrigues R, Magalhaes PK, Fernandes MI, et al. Neonatal screening for cystic fibrosis in São Paulo State, Brazil: a pilot study. Braz J Med Biol Res 2009;42:973-8.

20. Massie RJ, Olsen M, Glazner J, et al. Newborn screening for cystic fibrosis in Victoria: 10 years' experience (1989-1998). Med J Aust 2000;172:584-7.

21. Sarles J, Giorgi R, Berthézène P, et al. Neonatal screening for cystic fibrosis: comparing the performances of IRT/DNA and IRT/PAP. J Cyst Fibros 2014; 13: 384-90.

22. Sontag MK, Lee R, Wright D, Freedenberg D, Sagel SD. Improving the Sensitivity and Positive Predictive Value in a Cystic Fibrosis Newborn Screening Program Using a Repeat Immunoreactive Trypsinogen and Genetic Analysis. J Pediatr 2016; 175:150-58.

23. Sommerburg O, Krulisova V, Hammermann J, et al. Comparison of different IRT-PAP protocols to screen newborns for cystic fibrosis in three central European populations. J Cyst Fibros 2014; 13:15-23.

24. Yalçın EG. Yenidoğan taramasında neredeyiz? 8. Çocuk Solunum Yolu Hastalıkları ve Kistik Fibrozis Kongresi. 9-12 Mayıs 2018, Ankara. p 11. Available from http://www.cocuksolunum2018.org/

Table 1. The annual demographic characteristics of the newborn screening for cystic fibrosis in Mersin province.

	2015	**2016**	**2017**
Population of Mersin	1,745,221	1,773,852	1,793,931
Annual newborn birth	28,291	28,040	27,545
Annually screened newborns	27,969	27,030	27,274
Percentage of screened newborns	%98,86	%96,40	%99,02
NBSP* infants	138	217	157
Birth weight (grams)	3198±565**	3202±547**	3208±630**
	(790-4200)***	(1600-4900)***	(660-4910)***
Male gender	65 (47.1%)	108 (49.8%)	76 (48.4%)
Unachievable newborns	7 (%5.1)	17 (%7.8)	17 (%10.8)
Time of sweat test (days)	91,8±30,9**	53,8±31,2**	119,9±39,4**
	(15-214)***	(16-207)***	(18-201)***

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as: Özdemir A, Doğruel D. Newborn Screening for Cystic Fibrosis in Mersin Province: Yearly Assessment of the National Program. Turk Thorac J 2019; DOI: 10.5152/TurkThoracJ.2019.18187

Sweat test result			
Negative	115 (%83,3)	189 (%87,1)	129 (%82,2)
Intermediate	13 (%9,4)	8 (%3,0)	8 (%5,1)
Positive	3 (%2,2)	3 (%1,1)	3 (%1,9)
CFMS/CFSPID****	1 (%0.7)	2 (%0.9)	1 (%0.6)
Annual incidence of CF	1/9430	1/9347	1/9182

*NBSP: Newborn screen positive

**Data is expressed as mean±standard deviation

***Data is expressed as range (minimum-maximum)

****CFMS/ CFSPID: CFTR-related metabolic syndrome /cystic fibrosis screen positive inconclusive diagnosis

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as: Özdemir A, Doğruel D. Newborn Screening for Cystic Fibrosis in Mersin Province: Yearly Assessment of the National Program. Turk Thorac J 2019; DOI: 10.5152/TurkThoracJ.2019.18187