

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

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Abstract

OBJECTIVES: A national newborn screening program for cystic fibrosis (CF) was started 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 on NBSP infants were retrospectively analyzed between 2015 and 2017 from records of Mersin Women & Children's Hospital and Mersin City Training and Research Hospital.

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

KEYWORDS: Cystic fibrosis, newborn screening, immunoreactive trypsinogen test

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INTRODUCTION

Cystic fibrosis (CF) is an autosomal recessive disorder of the 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 in 80%-85% patients and recurrent lung infections result in an obstructive chronic lung disease that 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 of approximately 1/3000 [4]. Notably, one can assume that the rate is much higher as consanguineous marriages are common in Turkey.

Cystic fibrosis is caused by a genetic mutation, defined in 1989, in a gene on chromosome 7q31 that codes for a 1480-amino acid protein called "transmembrane conductance regulator" (CFTR) that functions as a transmembrane cAMP-activated chloride channel [5]. There are over 2000 different mutations in the CFTR gene that can cause diseases [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, delF508, is reported in 23.94% of CF patients in Turkey [7].

The newborn screening test for early diagnosis of CF disease was initially reported in the late 1970s with the measurement of IRT levels and gradually established mainly in North America, Oceanian countries, and Europe with various screening programs [8-10]. The main goal of newborn screening programs is to determine CF in infants at an earlier stage, appropriate follow-up in CF specialized care centers, and delay or prevent health problems related to CF. A retrospective study from Italy investigating the survival of infants diagnosed with newborn screen-positive (NBSP) or having CF-related symptoms between 1971 and 2014 had shown that 30-year survival was better in the NBSP group (80.1%) than in the CF-related symptom group (71.0%). Additionally, if the data were segregated for severe and moderate categories, the 20-year survival was significantly higher in the NBSP versus CF-related symptom group in the severe (85% vs. 64%) and moderate (94% vs. 86%) categories [11].

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Turkey has started a national newborn screening program using two repeated immunoreactive trypsinogen (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 acinar cells. Per the newborn screening policy for CF in Turkey, the first IRT test should be obtained in the first days of life on a dried blood spot specimen, and a cut-off value of $>90 \mu\text{g/L}$ is considered positive. Subsequently, the second IRT test should be obtained in IRT-positive newborns of the first test at the age of 7-14 days. At a level exceeding $70 \mu\text{g/L}$, newborns are referred to a specialized center for further investigation and sweat test procedure.

Our pediatric pulmonology center is the only affiliated hospital for referrals of NBSP babies in Mersin province since the implementation of the nationwide IRT/IRT program starting in January 2015. The initial referral center was Mersin Women & Children's Hospital between January 2015 and February 2016. Then, after the closure of this hospital, Mersin City Training & Research Hospital has been the center of NBSP referrals. The aim of this study was to evaluate the performance of a newborn screening program for CF in Mersin province based on the results of first 3 years.

MATERIALS AND METHODS

The records of Mersin Women & Children's Hospital and Mersin City Training & Research Hospital of all NBSP babies were retrospectively investigated between 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 referrals 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 induced by applying pilocarpine iontophoresis to infants' forearm by an experienced nurse. A sweat collection device was attached to one arm and a minimum amount of $\geq 75 \text{ mg}$ sweat was collected 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 $<30 \text{ mmol/L}$ was considered normal; $30\text{-}59 \text{ mmol/L}$ was defined as intermediate; $\geq 60 \text{ mmol/L}$ was considered positive for the diagnosis of CF in infants aged

<6 months. Sweat chloride level $<40 \text{ mmol/L}$ was considered normal; $40\text{-}59 \text{ mmol/L}$ was defined intermediate; $\geq 60 \text{ mmol/L}$ was considered positive for the diagnosis of CF in infants aged ≥ 6 months. 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 CF screen-positive inconclusive diagnosis (CFSPID).

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

Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences version 17.0 software statistical package program (SPSS Inc., Chicago, IL, USA). Continuous variables were expressed as mean and standard deviation 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 of the disease. The 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 diseases 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 newborns (screen coverage: 98.86% of born babies) in 2015, 27,030 newborns (screen coverage: 96.04% of born babies) in 2016, and 27,274 newborns (screen coverage: 99.02% of born babies) in 2017 were screened by the IRT/IRT test. There were 138 NBSP babies in 2015, 217 in 2016, and 157 NBSP in 2017 referred to our center for further evaluation. The weight and sex distribution of the NBSP babies were as follows: weight, $3198 \pm 565 \text{ g}$ (range: 790-4200 g) and male sex, 65 (47.1%) in 2015; weight, $3202 \pm 547 \text{ g}$ (range: 1600-4900 g) and male sex: 108 (49.8%) in 2016; and weight, $3208 \pm 630 \text{ g}$ (range: 660-4910 g) in 2017 and male gender: 76 (48.4%) (Table 1).

There were some NBSP babies who 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 2015, 17 babies (7.8%) in 2016, and 17 babies (10.8%) in 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 2015, 53.8 ± 31.2 days (range: 16-207 days) in 2016, and 119.9 ± 39.4 days (range: 18-201 days) in 2017. Sweat test result was normal in 115 (83.3%) infants in 2015, 189 (87.1%) infants in 2016, and 129 (82.2%) infants in 2017. Sweat test result in the intermediate range was reported in 13 (9.4%) infants for 2015, 8 (3.0%) infants for 2016, and 8 (5.1%) infants for 2017. The intermediate sweat test reported infants had a second sweat test at the age of 6 months. Among 29 infants, only 4 had consistent intermediate sweat test result and are considered CRMS/CFSPID. Sweat test result was positive in

MAIN POINTS

- The study illustrates that newborn screening program for cystic fibrosis is highly successful in Mersin province.
- IRT/IRT test is generally an effective method in defining cystic fibrosis among newborns.
- The incidence of cystic fibrosis is lower than expected in our region.

Table 1. 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 (g)	3198±565** (790-4200)***	3202±547** (1600-4900)***	3208±630** (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** (15-214)***	53.8±31.2** (16-207)***	119.9±39.4** (18-201)***
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

^aData are expressed as mean±standard deviation, ^bData are expressed as range (minimum–maximum)

NBSP: Newborn screen-positive; CFMS/CFSPID: CFTR-related metabolic syndrome/cystic fibrosis screen positive inconclusive diagnosis

3 (2.2%) infants in 2015, 3 (1.1%) infants in 2016, and 3 (1.9%) in 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 in 9,430 in 2015, 1 in 9,347 in 2016 and 1 in 9,182 in 2017, respectively. The overall incidence of the disease in Mersin was 1 in 9,388 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 was 90.0% and 99.4%, respectively.

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

DISCUSSION

The decision to include nationwide newborn screening for CF in Turkey has started on January 2015. Since then, our pediatric pulmonology section has been the referral center of NBSP babies. A total of 82,273 were screened by the IRT/IRT method in our region between 2015 and 2017. Our results showed that the newborn screening for CF in Mersin was highly successful. However, 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 limitations 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 conduct such an analysis due to lack of a 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 recognized for which the phenotypic consequences are unclear. The US centers proposed a term for designation of these infants, CFTR-related metabolic syndrome, and subsequently, European guidelines used the term CF 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 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 caregivers.

In general, the incidence of CF implemented by national neonatal screening program is surprisingly lower than that 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 of 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 the IRT/IRT protocol, the incidence of CF was reported 1 in 8,403 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 successful in detecting most babies with CF. The incidence reported was 1/2874. However, 9 of 191 cases of CF were missed by the IRT test [20].

Today, different methodologies are used to screen newborns for CF in different 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 the IRT/DNA method shows 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 United States evaluating the performance of IRT/IRT and IRT/DNA methodology as a newborn screening for CF had shown that CFTR-DNA panel sensitivity was 96.2% compared to 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 2018 and most CF centers have started recording their data to the system. The CF Registry is a secure centralized 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 Turkish 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 neighboring countries. Our center is initially joined to the national program and contributes the data of CF patients to the system from Mersin province.

To our knowledge, there have 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. Last, CF patients identified by screening will be followed by specialized CF centers, and many CF-associated problems with a late diagnosis can be prevented by early diagnosis and such an approach will 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 successful. The program has allowed early diagnosis of CF patients in our region.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Çukurova University (Approval No 2018/36).

Informed Consent: Due to the retrospective design of the study, informed consent was not taken.

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