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Evaluation of Patients Referred Following Cystic Fibrosis Newborn Screening: Four Year Experience of A Single-Center

Kistik Fibrozis Yenidoğan Taraması Sonrası Yönlendirilen Bebeklerin Değerlendirilmesi: Dört Yıllık Tek Merkez Deneyimi

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ABSTRACT

Objective: Cystic fibrosis (CF) is an inherited disorder which has negative impact on life span and quality. Newborn screening (NBS) is carried out by sweat chloride test (SCT) in babies with high immunoreactive trypsinogen (IRT) values. We aimed to present our data consisting of application and evaluation of sweat chloride test in babies referred to our center with two elevated IRT levels following national NBS.

Material and Methods: This retrospective study evaluated clinical data, sweat chloride test and CF transmembrane regulator (CFTR) gene mutation analysis results of referred infants due to positive CF NBS between January 2015–January 2019. Sweat chloride test results were interpreted as normal (<30mmol/L), intermediate (30-59 mmol/L) or high (≥60mmol/L). Infants missing a second test despite an initial invalid result (<10 mmol/L) or inadequate sweat collection were not included.

Results: The study group consisted of 184 infants of female/male ratio as 113/71(61.4/38.6%). The median admission time was postnatal 26 (9-98) days. The mean gestational week was 39 ± 1.75 weeks and mean birth weight was 3300 ± 432 grams. All infants passed their first stool in 48 hours at most. Five infants (3.6%) faced prolonged jaundice and two (1.4%) had neonatal pneumonia. Weight gain was insufficient in seven (5%) infants. No infant showed physical examination findings consistent with CF. First SCT was normal (n=153, 83.1%), intermediate (n=9, 4.9%) or high (n=1, 0.5%). Thirty-one infants had the second test because of inadequate sweat collection, invalid or high/intermediate range initial result. Consequently, 178 infants (96.7%) reflected normal results. Repeated SCT was high (n=2, 1.1%)/ intermediate (n=4, 2.2%) in six (3.3%) infants. CFTR mutation analysis revealed one homozygote F508 deletion and three 5T/7T/9T polymorphisms. Three patients received treatment while three were conservatively followed-up.

Conclusion: NBS can provide early diagnosis of patients with CF if standardized implementation and careful interpretation of sweat chloride test are achieved. Sharing of data by centers performing sweat chloride test is extremely important for the assurance of interoperability of the system.

Key Words: Cystic fibrosis, Newborn screening, Sweat chloride test

ÖZ

Amaç: Kistik fibrozis (KF), yaşam kalitesini ve süresini olumsuz etkileyen kalıtsal bir hastalıktır. Ülkemizde yenidoğan taraması topuk kanında bakılan immün reaktif tripsinojen (IRT) düzeyi yüksek bulunan bebeklerde ter testi (TT) yapılarak uygulanmaktadır. Bu çalışmada ulusal yenidoğan taramasında iki defa yüksek çıkan IRT nedeniyle merkezimize yönlendirilen bebeklerin TT yapılma ve değerlendirilme sürecinde elde ettiğimiz verileri sunmayı amaçladık.

Gereç ve Yöntemler: Bu retrospektif çalışmada Ocak 2015 - Ocak 2019 arasında merkezimize KF yenidoğan tarama testi pozitifliği nedeniyle yönlendirilen bebeklerin klinik verileri, TT ve kistik fibrozis transmembran regülatör (KFTR) geni mutasyon analizi sonuçları araştırıldı. Ter testi <30 mmol/L olanlar normal, 30-59 mmol/L olanlar şüpheli yüksek, ≥60 mmol/L olanlar yüksek olarak değerlendirildi. İlk TT<10 mmol/L olması veya yetersiz ter elde edilmesine rağmen ikinci TT yapılamayan bebekler çalışmaya dahil edilmedi.

Bulgular: Çalışma grubunu toplam 184 bebek [kız/erkek 113/71 (%61.4/%38.6)] oluşturdu. Başvuru süreleri ortancası postnatal 26 (9-98) gün, ortalama gestasyon haftası 39±1.75 hafta, ortalama doğum ağırlıkları 3300±432 gr'dı. Tamamı

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ilk dışkısını ilk 48 saat içinde yapmıştı. Beşinde (%3.6) uzamış sarılık, ikisinde (%1.4) yenidoğan pnömonisi vardı. Yedi bebeğin (%5) kilo alımı yetersizdi. Fizik incelemede KF bulgusu olan bebek yoktu. Toplam 153 bebeğin (%83.1) ilk TT sonucu normal, birinin (%0.5) yüksek, dokuzunun (%4.9) şüpheli yüksekti. Otuzbir hastada TT geçersiz sonuç, yetersiz ter miktarı, yüksek/şüpheli yüksek sonuç nedeniyle tekrarlandı. Sonuç olarak 178 bebeğin (%96.7) TT sonucu normaldi. Altı bebeğin (%3.3) ikinci TT yüksek (%1.1,n=2)/şüpheli yüksekti (%2.2,n=4). KFTR mutasyon analizinde bir bebekte F508del homozigot mutasyonu, üç bebekte 5T/7T/9T polimorfizmi saptandı. Bu bebeklerin üçüne tedavi başlanıldı, üçü tedavisiz izleme alındı.

Sonuç: Yenidoğan taraması ile KF hastalarının erken dönemde saptanılması ancak standardize TT uygulanması ve sonuçların dikkatli yorumlanması ile mümkün olacaktır. TT uygulayan merkezlerin TT yöntemlerini ve sonuçlarını içeren verileri paylaşması sistemin işlerliğinin kontrolü açısından önemlidir.

Anahtar Sözcükler: Kistik fibrozis, Yenidoğan taraması, Ter testi

INTRODUCTION

Cystic fibrosis (CF) is a life-shortening autosomal recessive (OR) disease which has negative impact on quality of life. It is caused by mutations in the gene which encodes CF transmembrane regulator (CFTR) protein located on the cell surface of all mucus producing organs in the body (1). To date, more than 2000 mutations of CFTR protein, which plays a role in the epithelial chloride transport, have been identified (2). The estimated incidence of the disease in whites is 1/2.000-1/3.500 and carrier frequency is reported as 1/25 (3). As seen in other OR inherited diseases, its incidence is higher in countries including ours with high consanguinity. Based on the limited number of studies performed before incorporation of CF into the NBS program, the incidence was reported as 1/3,000 (4,5) in Turkey. The disease doesn't show gender predominance.

Cystic fibrosis is a multisystem disorder which may affect all the exocrin glands in the body. It causes chronic obstructive lung disease, malabsorption and malnutrition due to pancreatic insufficiency, hepatic insufficiency, cirrhosis, diabetes mellitus and defects of the reproductive system (6). The most frequently affected organ is the lung and mortality is due to respiratory insufficiency in more than 90% of patients (7).

Diagnosis criteria for CF developed by CF Foundation have been revised in 2015 (8). A positive NBS or signs suggestive of CF or positive family history of CF in a parent or sibling will be diagnosed with CF if at least one of the following criteria is met: A sweat chloride level ≥60 mmol/L or indentification of 2 CF-causing mutations or nasal potential difference measurement consistent with CF.

The current median survival age for CF has risen to 40 years from less than two years at the time of initial description of the disease in 1938 (9). This improvement in survival is due to widespread implementation of NBS programs, modification of standard treatment modalities and new treatment strategies. Implementation of NBS in many countries with a high incidence of CF has positive effects such as prevention of progressive lung injury and malnutrition in addition to increased survival age (10-12). NBS has been implemented in our country since January 1st,2015. For this purpose, immunoreactive tripsinogen (IRT), which acts as an indicator of pancreatic damage, is measured from dried blood samples obtained on the 3rd-5th days of life. This measurement is repeated if IRT level is higher than expected (13). Newborns with two high IRT levels are referred to centers performing sweat chloride test. Turkish Ministry of Health has published a guide called "Guide for monitorization of patients diagnosed with CF by newborn screening" (14). Infants with a high or intermediate range sweat chloride test should be referred to a CF center, and receive necessary follow-up and treatment. CFTR mutation analysis is especially helpful for infants with intermediate range (30-59 mmol/L) sweat chloride values.

Herein, we aimed to present our data consisting of application and evaluation of SCT in babies referred to our center with two elevated IRT levels following national NBS.

MATERIALS and METHODS

Records of infants referred to Department of Pediatric Pulmonology, Ankara University School of Medicine between January 2015-January 2019 for sweat chloride test due to positive CF NBS were evaluated in this retrospective study. The study protocol was approved by the ethical committee of our university (Study approval number:02-109-19; Jan 2019). Infants missing a second test despite an initial invalid result (<10 mmol/L) or inadequate sweat collection were not included.

Age at referral, gender, time of passage of first stool, signs and symptoms suggesting CF, gestational age, birth weight, consanguinity between parents and family history of infant loss were searched and recorded. Sweat chloride test values and CFTR gene mutation analysis, if available, were evaluated.

Sweat chloride test and its interpretation:

Sweat chloride test, based on the measurement of chloride value in sweat, remains the gold standard for the diagnosis of CF. This test was developed by Gibson and colleagues in 1959 (15). It is being performed in accordance with recommendations of "National Cystic Fibrosis Newborn Screening Program - Sweat Chloride Test Guide", released by Turkish Ministry of Health, Turkish Public Institution of Health in our country (5). Sweat chloride testing can be performed in infants more than 36 weeks of gestational age, 2 kg and 10 days of age for the collection of adequate amount of sweat. Sweat chloride test is applied using pilocarpine iontophoresis" (UTSAT CF

Collection System) technique in our center. Both conductivity and sweat chloride measurement are achieved by this method. Pilocarpine containing gels are placed on electrodes, fixed on a small area on the forearm and low dose electric current is applied. The stimuated area is dried following five minutes of this iontophoresis procedure. A macroduct collector is placed to this area, and sweat is collected for 25-30 minutes. Sweat is analyzed at the last phase. Electrical conductivity of sweat is measured for this procedure. Conductivity is an indirect measurement which reflects the total amount of many electrolytes in sweat. In addition, sweat chloride values are also calculated in our center.

A high sweat chloride value in an infant with a positive NBS is consistent with a diagnosis of CF. An infant with a positive NBS or signs suggestive of CF or positive family history of CF in a parent or sibling will be diagnosed with CF if sweat chloride value is ≥60 mmol/L, but a repeated sweat chloride test is recommended for a definite diagnosis. A diagnosis of CF is excluded in an infant with positive NBS and a sweat chloride test value <30 mmol/L unless clinical signs suggestive of CF are present. Sweat chloride test is repeated in infants with a positive NBS or signs suggestive of CF or positive family history of CF in a parent or sibling and a sweat chloride test value 30-59 mmol/L when the infant is two months of age. A thorough clinical evaluation and CFTR mutation analysis should de considered if the second test is also in the intermediate range. If 0 or 1 CF-related mutations are identified, the infant will be diagnosed with "CFTR-related metabolic syndrome" (CRMS) or "CF screen-positive, inconclusive diagnosis" (CFSPID) (14).

Statistical analysis:

Statistical analysis was performed using the SPSS statistical package (v.18.0). Counts and percentages were reported for categorical variables. For continuous variables, mean±SD were used for normal distribution, while median values and ranges were used for non-normal distribution. Comparison analysis and hypothesis testing were not made as it was designed as a descriptive study.

RESULTS

Demographic and clinical findings:

A total of 229 infants were referred to our center between January 2015-January 2019 as a result of positive NBS. Sweat chloride test was administered to all. Infants missing a second test despite an initial invalid result (<10 mmol/L) n=33) or inadequate sweat collection (n=12) were not included (Figure 1). As a result, the study group consisted of 184 infants [female/male 113/71(61.4%/38.6%)]. The median admission time was postnatal 26 (9-98) days and 50 infants (27.2%) have admitted beyond postnatal 30 days.

Demographic and clinical data of 45 referred infants with only sweat chloride values recorded but clinical evaluation missing were excluded. The mean gestational age of 139 infants with adequate file information was 39±1.75 weeks. Corrected gestational age was < 36 weeks in only one infant (353 weeks). Mean birth weight was 3300±432 gr. Time of passage of first stool was 24 hours in 136 infants (97.8%), and between 24-48 hours in 3 infants (2.2%). Nine infants (6.5%) were admitted to the neonatal intensive care unit in the neonatal period due to respiratory distress. Five infants (3.6%) faced prolonged jaundice and two (1.4%) had a history of neonatal pneumonia. Cough (n=2, 1.4%), diarrhea (n=1, 0.7%) and salty skin taste (n=1, 0.7%) were other rare symptoms. Wheezing, nasal discharge and history of meconium ileus were not reported. Parental consanguinity was positive in 12 infants (8.6%). Family history of CF or infant death were not positive for any of the infants. Weight gain was insufficient in seven (5%) infants. Signs suggestive of CF were considered in physical examination. Rales, rhonchi, retractions, rectal prolapse or edema suggestive of hypoalbuminemia were not mentioned in any of the infants.

Sweat chloride test results:

Sweat chloride test results are given in Figure-1. First sweat chloride test was normal in a total of 153 infants (83.1%). The initial test was high (≥60 mmol/L) in 1 infant (0.5%) and intermediate (30-59 mmol/L) in 9 infants (4.9%). Thirty-one infants had the second test in two weeks because of

Table I: The summary of patients on follow-up with a diagnosis or suspicion of cystic fibrosis (CF).

Patients	1.st sweat chloride test (mmol/L)	2. nd sweat chloride test (mmol/L)	CFTR* protein mutation analysis	Treatment
1	Inadequate amount of sweat	77	Homozygote F508 deletion, 5T/7T/9T polymorphism (9T allele)	Yes
2	Inadequate amount of sweat	32 and 50	Result pending	No
3	40	43	5T/7T/9T polymorphism (5T/7T allele)	No
4	79	85.5	5T/7T/9T polymorphism (7T allele)	Yes
5	48	56	Result pending	Yes
6	31	34	Result pending	No

***CFTR:** *Cystic fibrosis transmembrane regulator*

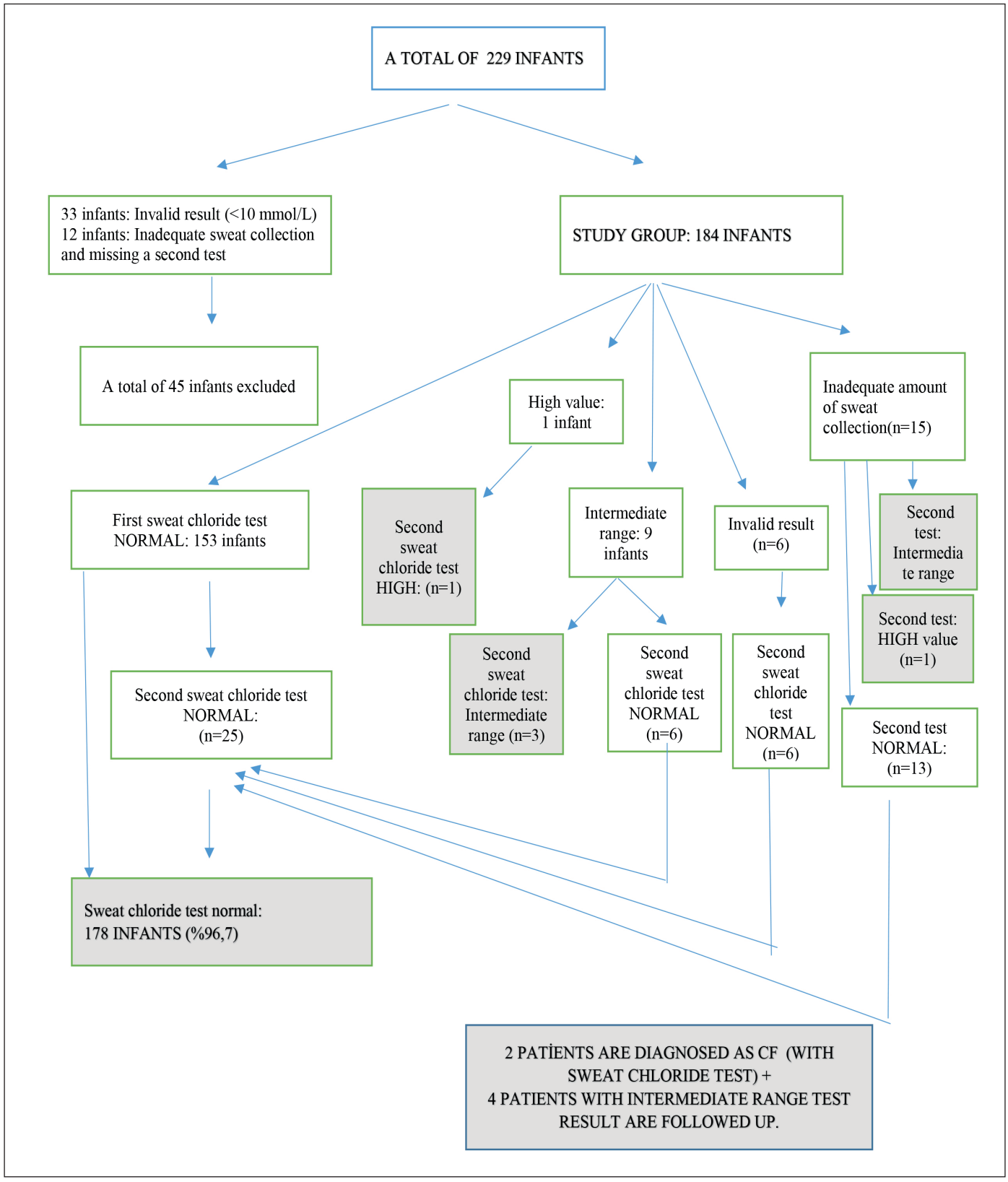

Figure 1: Sweat chloride test results of referred infants

inadequate amount of sweat collection (n=15), invalid (n=6) or high/intermediate range (n=10) initial result. One infant with a high value in the initial sweat chloride test also had a high value in the second test. The second sweat chloride test of 9 infants with an intermediate test value revealed normal in 6 and an intermediate value in 3 of them. Amongst 15 infants with inadequate amount of sweat collection in the initial sweat chloride test, second test resulted with a normal value in 13 infants while one infant showed a high value and one infant had an intermediate value. The repeated test result of six infants with an invalid result (<10 mmol/L) was normal in all. Consequently, 178 infants (96.7%) reflected normal results. In a total of 6 (3.3%) infants, repeated sweat chloride test was high (n=2, 1.1%) or intermediate (n=4, 2.2%), and follow-up was decided for these infants.

Patients followed-up with a diagnosis or suspicion of CF:

The summary of patients on follow-up with a diagnosis or suspicion of cystic fibrosis (CF) is seen in Table 1. Consangiuinity between parents was present in one of these infants. None of the infants had a positive familial history of infant loss or CF. All of them have passed their first stool in the first 24 hours. One infant passed through prolonged jaundice and one had respiratory distress in the neonatal period. Two infants were diagnosed with CF at postnatal days 31. and 36. as a result of high value (≥ 60 mmol/L) in the repeated sweat chloride test. The remaining four patients had an intermediate value. CFTR mutation analysis was studied in all patients who were followed. One patient had homozygote F508 deletion and three patients had 5T/7T/9T polymorphisms. Treatment consisting of pancreatic enzyme extract, vitamins A,D,E and K, and dornase alfa were initiated to two patients who were diagnosed with CF as a result of high value in the sweat chloride test. Four patients with an intermediate test value were conservatively followed-up. On the follow-up, chronic treatment was initiated to one of these patients who did not receive any treatment initially, as a result of repeated lower respiratory tract infection on the follow-up. Of these patients, four of them are still followed by both pediatric pulmonology and hepatology and nutrition departments.

DISCUSSION

Newborn screening tests present amongst preventive health services which possess significant importance in the context of public health programs throughout the world. NBS tests are implemented for early diagnosis of relatively often encountered diseases in a defined population, which have cost-effective screening methods, appropriate confirmatory test if screening is positive, and acceptable treatment or control of the clinical course following diagnosis. In this context, we aimed to draw attention to CF NBS, the latest included screening to our NBS program, by presenting the data of our center.

The IRT test, first established in 1979, has been developed to provide diagnosis of CF in the first weeks of life, before the presentation of clinical signs and symptoms (16). Currently, NBS is implemented in many countries including our country. The diagnosis of CF initially relied basically on clinical symptoms and signs before the introduction of NBS, while currently at least 64% of new diagnoses in the US consist of asymptomatic infants following a positive NBS (17). Approximately 1000 new cases are diagnosed with CF annually in the US. NBS program is the main cause of diagnosis of 70% of these infants in the first 2 years of life (6). Similarly, UK CF Registry notifies a diagnosis of 180 infants in 2016 by NBS (18). A total of 140-150 infants are being diagnosed with CF by NBS annually since 2015 in our country, presenting with a birth rate of approximately 1.300.000 infants per year, according to The General Directorate of Public Health Department of Child and Adolescent Health Report. It is believed that the large majority of patients diagnosed with CF will consist of infants detected by NBS program in the previous years. Nevertheless, some individuals are diagnosed symptomatically even in regions administering NBS, either because of birth before the incorporation of CF NBS or false-negative screening results. For this reason, clinical signs and symptoms of CF must be well known by clinicians, and infants presenting with suggestive signs should be evaluated regardless of the NBS results (11).

The IRT cut-offs are generally set such that 1-5% of all sample values are resulted as positive in the initial test in order to optimize the sensitivity and positive predictive value of this screening method. Moreover, conditions such as prematurity and perinatal distress increase the likelihood of false-negative results. Therefore, repetition in infants with positive initial results increase the positive predictive value of this test (11).

Diagnosis in the first weeks of life before the appearance of signs is possible if infants with two positive IRT values are referred on time to centers implementing sweat chloride test (19).

The cut-off value is accepted as 90 ng/ml for the first and 70 ng/ml for the second sweat chloride test. Similarly, our cases did not exhibit signs and symptoms suggestive of CF. Even though NBS aims diagnosis and initiation of the treatment by at most 1 month of age, 2013 CF NBS Registry data in US shows that the median age of referral of infants to a CF center was beyond postnatal 30 days in 39 states, and > 42 days in 21 states (20). Similarly, 50 (27.2%) of patients in our study group have admitted to our center after postnatal day 30. Two of our patients with CF were also diagnosed later than postnatal 30 days. This finding delineates the neccessity of rapid assessment of CF screening results, rapid reach to infants with a positive screening and implementation of a second test, family explanation and referral to a center where sweat chloride test is performed in case of a second positive test result.

Correct implementation and interpretation of sweat chloride test, which is accepted as the gold standard for the diagnosis of CF, by an experienced team is crucial. Repetition of the test following enough hydration at rest is mandatory in patients with inadequate amount of sweat collection. Our series also

reflected inadequate material in 8.2% (n=15). Repetition of the test revealed a high result in one patient and an intermediate result in one patient. Repetition of sweat chloride test is also advocated if the result indicates <10 mmol/L or >160 mmol/L (7). The repeated test results were normal in six patients with an initial low test result.

Based on the CF Foundation Consensus Guideline which was last updated in 2017, a diagnosis of CF is excluded in newborns with a sweat chloride test result <30 mmol/L performed due to positive IRT and without symptoms of CF or a family history of CF (8). We concluded that a diagnosis of CF is unlikely in 96.7% of our series because of a normal sweat chloride test and no family history or symptoms of CF. The same guideline notifies that infants with an intermediate range (30-59 mmol/L) value in two sweat chloride tests should undergo CFTR gene analysis, and close monitorization by pediatric pulmonology department should be provided. These patients are diagnosed with CF if 2 CF-related mutations exist while a diagnosis of CFTR-related metabolic syndrome (CRMS) is likely in case of 0-1 mutations. Follow-up is provided for these patients subsequently. These patients generally exhibit normal pancreatic functions and growth whereas Pseudomonas-positive oropharngeal culture rates are reported as 10.7-78.4% (11). Patients considered as CRMS should be followed on a regular basis. A diagnosis of CF can be made in these patients on the follow-up in case of appearance of clinical signs, a repeated sweat chloride value ≥60 mmol/L or determination of CF-related mutations by a detailed genetic evaluation. This rate was reported as 8-48 % in a systematic review of Grooves et al. which included studies performed in this area (21). Repeated sweat chloride test of four patients in our series revealed intermediate values. CFTR gene analysis was made in all of these patients. To date, only one patient's analysis has resulted yet,- and signified 5T/7T/9T polymorphism (5T/7T allele). The presence of this polymorphism is reported as 10% in the population (22). This patient was decided to be followed without treatment as positive clinical findings or family history were not present. On the follow-up, treatment was initiated to one patient with repeated lower respiratory tract infections and an intermediate sweat chloride test result whose genetic testing has not resulted yet.

CF Foundation suggests a diagnosis of CF if the sweat chloride value is ≥ 60 mmol/L, and performance of CFTR gene mutation analysis in these patients (8). Similarly, one of our two patients with a high value of sweat chloride test had homozygote F508 deletion while the other patient displayed 5T/7T/9T polymorphism. Treatment was initiated to both of our patients and follow-up was planned.

This study has several limitations. Firstly, our data includes single center data and our study group consist of a limited number of patients. Conduction of multi-center studies and investigation of complete national data will enlighten clinicians better in regarding steps to be taken following positive CF NBS. The second limitation was the deficiency of clinical data in patient records. Lastly, adequate long-term follow-up findings of patients could not be collected as this study covered a period of four years.

CONCLUSION

CF is an inherited disorder with a relatively higher incidence in countries like ours presenting with high consanguinity. NBS can provide early diagnosis of patients with CF if standardized implementation and careful interpretation of sweat chloride test are achieved. Sharing of data by centers performing sweat chloride test is extremely important for the assurance of interoperability of the system. Future research reflecting complete, long-term national data will provide singificant contribution regarding incidence, diagnosis and long-term prognosis of CF in our country.

Ethical approval: The study protocol was approved by the Ankara University School of Medicine Institutional Ethics Committee (Study approval number:02-109-19; Jan 2019).

Conflict of interest: The authors declare no conflict of interest.

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