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*Case Report*

# Normal sweat chloride test does not rule out cystic fibrosis

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**SUMMARY: Başaran AE, Karataş-Torun N, Maslak İC, Bingöl A, Alper ÖM. Normal sweat chloride test does not rule out cystic fibrosis. Turk J Pediatr 2017; 59: 68-70.**

**A 5-month-old patient presented with complaints of fever and cough. He was hospitalized with the diagnosis of bronchopneumonia and pseudo-Bartter's syndrome. Patient was further investigated for diagnosis of cystic fibrosis. The chloride (Cl) level in sweat was determined within the normal range (25.1 mmol/L, 20.3 mmol/L). CFTR (Cystic Fibrosis Transmembrane Regulator gene; NM_000492.2) genotyping results were positive for p.E92K; p.F1052V mutations. The patient was diagnosed with cystic fibrosis. In our patient, with features of CF and normal sweat test, mutation analysis was helpful for the diagnosis of cystic fibrosis.**

***Key words: pseudo-Bartter's syndrome, cystic fibrosis, sweat test false negativity.***

Nowadays, measurement of chloride level in sweat (sweat test) is the most widely used biochemical method for the diagnosis of cystic fibrosis (CF; OMIM 219700). However, there are various reports in literature, regarding false positive or false negative sweat test results in CF patients. Therefore, the interpretation of sweat test results should be done by taking into consideration the clinical condition of the patient and possible accompanying comorbid situations. One of the reasons leading to the negative results on sweat test is the presence of specific mutations in CF patients[1,2]. In this article, we report a five-month-old boy who was diagnosed as CF with the findings of pseudo-Bartter's syndrome and compound heterozygous for p.E92K/p.F1052V mutations, but with negative for sweat test.

## Case Report

A 5-month-old male patient presented to hospital with complaints of fever and cough. The patient did not have a meconium ileus history. During the neonatal period he was hospitalized for four days to receive phototherapy and helmet oxygen therapy due to tachypnea and high indirect bilirubin. During the first three months of his life he had gained 10-15 grams per day and later the weight gain had decreased and he had lost 500 grams in the last 1.5 months before admission to the hospital. The newborn screening test for CF was in the normal range (immunoreactive trypsinogen level 39.5 ng/ml). His mother and father were not relatives and he had two healthy siblings. The anthropometric measurements of the patient were recorded as height 61 cm ($10^{th}$ percentile), head circumference 40.5 cm ($10^{th}$ percentile) and body weight 5280 gram (3-$10^{th}$ percentile). On physical examination, he had dry oral mucosa, sunken eyes and crepitant rales in right basal lung. A chest radiograph revealed right paracardiac infiltration. He was admitted to hospital with a diagnosis of bronchopneumonia. Laboratory examinations revealed metabolic alkalosis (pH 7.53, $pCO_2$ 38.6 mmHg, $HCO_3$ 32.1 mEq/L, base excess 8.9 meq/L), hyponatremia and hypochloremia (serum sodium 133 mEq/L, Cl 88.6 mEq/L). Total serum protein and serum albumin levels were in normal range. The patient was investigated for the etiology of hyponatremic hypochloremic metabolic alkalosis and he was diagnosed as pseudo-

Bartter's syndrome (OMIM 607364) with the findings of hyponatremia, hypochloremia and low urine Cl level (urine Cl: 21.6 mEq/L). Chloride level in sweat test was measured with The CFΔ Collection System® sweat test analysis system (UCF 2010 Iontophoresis Unit and UCF 2011 Sweat Analysis Unit), which is used to analyze both the conductivity and chloride concentration of sweat via conductivity measurement by a colorimetric end point software method; chloride level of our patient was 25.1 mmol/L (normal: 0-30 mmol/L, sample volume was 17$\mu$L). One month later, the chloride level in sweat, re-measured using the same method, was 20.3 mmol/L (sample volume was 18 $\mu$L). The *CFTR* genotyping was performed with the method of direct sequencing analysis: compound heterozygous genotype was detected in the patient, and parental carrier testing was confirmed. The patient was diagnosed with CF due to the clinical manifestation of CF and DNA analysis which revealed a compound heterozygosity (p.E92K / p.F1052V). Fecal elastasis level was detected as >200 $\mu$g/gram (normal range: >200 $\mu$g/gram). There was no microorganism detected in deep throat culture. His electrolytes were normal during follow up with ¼ teaspoon of sodium supply daily.

**Discussion**

Cystic fibrosis is an autosomal recessive disease, which results from a mutation in the transmembrane conductor regulator gene. The frequency of cystic fibrosis was reported as 1/3000 in Turkey in limited studies; however, it is thought that this ratio is higher due to the consanguinity[3]. In a study conducted in Turkey, it was shown that 7.1% of children who were admitted to hospital due to recurrent lung infection and/or malnutrition, had cystic fibrosis[4]. In addition, according to another study published in Turkey, pseudo-Bartter syndrome was detected in 12% of children who were diagnosed with cystic fibrosis[5].

Currently, the sweat chloride test remains the gold standard method for the diagnosis of cystic fibrosis[6]. The measurement of chloride in sweat is performed by chloridometer which performs colorimetric titration and is advised by Clinical and Laboratory Standards Institute (CLSI)[7]. For babies younger than 6 months old: the chloride level in sweat <30 mmol/L is normal, 30-59 mmol/L is intermediate value and ≥60 mmol/L is pathologically high. However, for babies older than 6 months and for adults: the chloride level in sweat <40 mmol/L is normal, 40-59 mmol/L is intermediate value and ≥60 mmol/L is pathologically high[7,8].

Cystic fibrosis caused by p.E92K is rare; based on CFTR2 database, only 33 patients have this mutation[9]. The p.E92K (c.274G>A; rs121908751) mutation in exon 4 was first described in two patients by Nunes et al.[10] in 1993. One of the patients whom they explored the p.E92K mutation originated from Spain (p.E92K/unknown, Cl in sweat 92 mmol/L) and the other patient was from Turkey (p.E92K/p.E92K, Cl in sweat 95 mmol/L). It is stated that, p.E92K mutation should be screened during the analysis of CF chromosomes in patients from Anatolia [10]. In 2008, only one Dutch patient was reported with this homozygous mutation[11]. In the literature, the frequency of this mutation was reported as 0.1% for Spanish and 66.6% in Chuvash patients[12, 13]. This missense mutation belongs to Class II type and occurs as a result of replacement of glutamic acid with lysine on codon 92 in exon 4 of the *CFTR* gene, at the first ectoplasmic loop of the CFTR protein. As stated by Stanke et al.,[11] the integrity of the loop at this position seems to be essential for the reabsorption of salt from the sweat duct, but less relevant for ion secretion in airways and intestine.

Little is known about the clinical phenotype of p.E92K mutation; we were not aware of a similar case in the literature; i.e. normal sweat test results with diagnosis of pseudo-Bartter's syndrome, in addition to growth retardation and lung infection.

p.F1052V mutation, (c.3154T>G; rs150212784) located in exon 17B of the *CFTR* gene, is also rare. Only 33 patients with this mutation in the CFTR2 database were reported, similar as p.E92K mutation. This mutation was first described by Mercier et al.[14] in 1993 in one patient. Onay et al.[15] has found the frequency of p.F1052V mutation as 1.2% in Turkish CF patients. Based on 2015 CFTR2 gene database; p.E92K mutation was classified in CF-causing variant while p.F1052V mutation was included in various clinical consequence mutations[8]. The mutation leads to the change of a phenylalanine

to a valine at the second transmembrane span of the CFTR protein. This change is expected to disturb the second hydrophobic transmembrane structure. Interestingly, no association was found between p.F1052V mutation and the sweat test in the literature.

Sweat test has been used as a routine laboratory test for approximately 50 years. During this period, various reports regarding the false positive or false negative sweat test results of cases were reported in literature. The results of sweat test of our patient were found to be among normal range the test was repeated twice. The *CFTR* genotyping analysis was detected compound heterozygote mutations for p.E92K/p.F1052V and diagnosis was confirmed with the clinical findings. We speculate that these mutations may lead to negative results on sweat test. In the literature, the causes of the false negative results on sweat test are indicated as technical reasons, specific mutations, hypohydrotic ectodermal dysplasia, inadequate sweat collection, mineralocorticoid treatment, young age, edema, hypoproteinemia and penicillin treatment. The first four reasons are the most common reasons and this specific mutation may be responsible for our negative sweat test result. Up to date, based on genotype-phenotype correlations, we are aware that heterogenous *CFTR* mutations can cause unusual electrophysiological or clinical manifestations.

In conclusion although the sweat chloride test is the most commonly used method for the diagnosis of cystic fibrosis, it does not always give a clear answer. Mutation analysis may be helpful when clinical findings are suggestive of cystic fibrosis, even if sweat test results are negative.

## REFERENCES


1. Highsmith WE, Burch LH, Zhou Z, et al. A novel mutation in the cystic fibrosis gene in patients with pulmonary disease but normal sweat chloride concentrations. N Engl J Med 1994; 331: 974-980.
2. Castellani C, Tamanini A, Mastella G. Protracted neonatal hypertrypsinogenaemia, normal sweat chloride, and cystic fibrosis. Arch Dis Child 2000; 82: 481-482.
3. Kiper N, Yalçın E. Kistik fibrozis. Sürekli Tıp Eğitim Dergisi 2003;12:131-33.
4. Cesur Y, Doğan M, Arıyuca S ve ark. Hastaneye başvuran malnutrisyonu ve/veya tekrarlayan akciğer enfeksiyonu olan çocuklarda kistik fibrozis sıklığı araştırılması. Selçuk Üniversitesi Tıp Dergisi 2010; 26: 138-141.
5. Bekem-Soylu Ö, Öztürk Y, Çetinkaya H ve ark. Kistik fibrozisli olgularda gastrointestinal tutulum ve nutrisyonel durumun değerlendirilmesi: Dokuz Eylül Çocuk Gastroenteroloji, Beslenme ve Metabolizma Ünitesi deneyimi. Turkiye Klinikleri J Pediatr Sci 2005; 1: 15-17.
6. Farrell PM, Rosenstein BJ, White TB, et al. Guidelines for diagnosis of cystic fibrosis in newborns through older adults: Cystic Fibrosis Foundation consensus report. J Pediatr 2008; 153: S4–S14.
7. Clinical and Laboratory Standards Institute. Sweat testing: sample collection and quantitative chloride analysis; approved guideline (3rd ed). Wayne, PA: Clinical and Laboratory Standards Institute, 2009.
8. Emiralioğlu N, Özçelik U, Yalçın E, Doğru D, Kiper N. Diagnosis of cystic fibrosis with chloride meter (Sherwood M926S chloride analyzer®) and sweat test analysis system (CFΔ collection system®) compared to the Gibson Cooke method. Turk J Pediatr 2016; 58: 27-33.
9. The Clinical and Functional Translation of CFTR (CFTR2). Available at URL http://cftr2.org (Accessed 26 July 2016)
10. Nunes V, Chillón M, Dörk T, Tümmler B, Casals T, Estivill X. A new missense mutation (E92K) in the first transmembrane domain of the CFTR gene causes a benign cystic fibrosis phenotype. Hum Mol Genet 1993; 2: 79-80.
11. Stanke F, Ballmann M, Bronsveld I, et al. Diversity of the basic defect of homozygous CFTR Mutation genotypes in humans. J Med Genet 2008; 45: 47-54.
12. Casals T, Gimnez J, Ramos MD, et al. Analysis of the CFTR Gene Confirms the High Genetic Heterogeneity of the Spanish Population: 43 Mutations Account for Only 78% of CF Chromosomes. Hum Genet 1994; 93: 447-451.
13. Stepanova AA, Abrukova AV, Savaskina EN, et al. Mutation p.E92K is the primary cause of cystic fibrosis in Chuvashes. Genetika 2012; 48: 863-871.
14. Mercier B, Lissens W, Novelli G, et al. Identification of eight novel mutations in a collaborative analysis of a part of the second transmembrane domain of the CFTR gene. Genomics 1993;16: 296-297.
15. Onay T, Zielenski J, Topaloglu O, et al. Cystic fibrosis mutations and associated haplotypes in Turkish cystic fibrosis patients. Hum Biol 2001; 73: 191-203.