Sweat chloride concentration in cystic fibrosis patients with cystic fibrosis trans-membrane conductance regulator 11234V mutation

To the Editor

I read with interest the recently published outstanding study by Abdul-Wahab et al[1] on the sweat chloride concentration in cystic fibrosis patients with cystic fibrosis trans-membrane conductance regulator 11234V mutation. Early and accurate diagnosis of cystic fibrosis (CF) is crucial to alleviate parental distress and allow earlier therapeutic intervention and genetic counseling. I have 3 comments on the aforementioned study.

First, for nearly more than 5 decades, the diagnosis of CF has relied upon the measurement of raised sweat chloride concentration (>60 mEq/L). While the validity of this test is universally accepted, increasing diagnostic challenges and the search for adequate biomarker assays to support curative oriented clinical drug trials have created a new demand for accurate, reliable, and more practical CF tests. It is noteworthy to mention that sweat testing, however, is cumbersome to the patient, prone to technical difficulties, and unreliable in young children <4 weeks as well as in adults because of increasing chloride concentrations with age. False-positive and false-negative results do exist.[2] Based on sweat chloride concentration and in an agreement with previously published studies,[3-5] Abdul-Wahab et al[1] stated in their study that both the original quantitative pilocarpine iontophoresis test (QPIT) and Wescore Macroduct sweat collector (WMSC) method were highly reliable procedures. And diagnostically, both were statistically equivalent in diagnosing CF. Truly, some concerns are nowadays triggered considering the "golden rule of 60" in precisely diagnosing CF particularly in borderline sweat chloride concentration. In a recent Italian study,[6] the relationship between CF trans-membrane conductance regulator gene (CFTR) mutation analysis and sweat chloride concentration was investigated in a cohort of subjects with borderline sweat test values, to identify misdiagnosis of CF. The mean value of sweat chloride concentration in the deoxyribonucleic acid (DNA) negative subjects were lower than in those with at least one CFTR mutation. The study concluded that sweat chloride concentration of 39 mEq/L is the best sensitivity trade-off for the sweat test on genotype.

Second, measuring sweat conductivity is a well-known diagnostic tool in CF. Abdul-Wahab et al[1] did not state in their study the conductance cut-off value applied in their study. I presume that they referred to the standard addressed by Lezana et al[7] study where they found that the best conductivity cut-off value for diagnosing CF being >90 mmol/L, and the best conductivity cut-off value to exclude CF being <75 mmol/L. Over-reliance on that standard might be unable to precisely discriminate those with CF from those with non-CF. Recently, Nanoduct (Giangarlo Scientific Co, Pittsburg, USA), an analyzing system measuring conductivity, which require only 3 microliters of sweat and gives results within 30 minutes, has been assessed in a Swiss[8] study over 3 years period on 1,041 subjects. In 95 children, Nanoduct failed (9.1% failure rate), mainly due to failures in preterm babies and newborns. Assuming 59 mmol/L as an upper limit of normal conductivity, 46 CF patients were correctly diagnosed (sensitivity 100%, 95% CI: 93.1-100; negative predicted value 100%, 95% CI: 99.6-100) and only 39 non-CF were false positive (specificity 95.7%, 95% CI: 94.2-96.9; positive predicted value 54.1%, 95% CI: 43.4-65.0). On increasing diagnostic limit of conductivity to 80 mmol/L, the failure rate fell to 0.3%. Cystic fibrosis patients had a median conductivity of 115 mmol/L; the non-CF a median of 37 mmol/L. Therefore, considering these conductance cut-off values will augment the reliability of Nanoduct test as a diagnostic tool for CF diagnosis and renders it a simple bedside test for fast and reliable exclusion, diagnosis, or suspicion of CF. In cases with borderline conductivity (60-80 mmol/L), other additional methods (determination of sweat chloride concentration and genotyping) might be needed.

Third, in comparison with both sweat chloride determination and sweat conductivity, cloning of the CF gene and the simultaneous identification of the predominant mutation remains the critical cornerstone criteria in the diagnosis of CF. Because of the high negative predictive value of DNA testing, in combination with its speed, reliability, and convenience for the patient, starting the diagnostic work-up for CF with DNA testing can be justified in hospitals which possess the laboratory facilities for this type of test.[2] The ability to test for CFTR mutations at the molecular level has already improved the diagnosis of symptomatic patients and expanded the reproductive options of family members of CF patients. The same technology also holds a promise of identifying asymptomatic carriers and at-risk couples without family history in the general population so that they too might be offered prenatal diagnosis or other options.[9] However, there are 2 major obstacles to genetic study, which might trigger ethical and public health concerns: 1) unidentified mutations are still numerous, except in certain populations. More than 1500 mutations have been identified in the CF CFTR gene, not all which result in CF, 2) the larger the number of tested mutations (and thus the better the efficiency of genetic surveillance), the more expensive the procedure.[10] Establishment of more advanced, but

cheaper genetic technologies are, therefore, fundamental for firm diagnosis of CF.

Mahmood D. Al-Mendalawi
Department of Pediatric
Al-Kindy College of Medicine
Baghdad University
Baghdad, Iraq

Reply from the Author

We appreciate Prof. Al-Mendalawi's comments. Actually, the subjects in our study are confined to CF patients CFTR I1234V mutation belonging to a large Arabic tribal family, and all subjects are the homozygous status.[11] The first objective of the present study was to define the pathological range of chloride level in a sweat test using the gold standard quantitative pilocarpine iontophoresis test using Gibson-Cooke method, and the second objective was to compare the Gibson-Cooke method with the Wescor Macroduct Sweat Collection System (WMCS) for measuring chloride concentration. Sweat conductivity was measured from sweat collected using WMCS. Generally, a diagnosis of CF can be made in a patient with clinical features of the disease if the concentration of chloride in sweat is >60 mmol/L, or if it is in the intermediate range (30-59 mmol/L for infants >6 months of age, 40-59 mmol/L for older individuals), and 2 disease-causing CFTR mutations are identified.[12,13]

Sweat chloride concentration increases with age in people without CF; however, a concentration greater than 60 mmol/L is still diagnostic of the disease.[14] Appropriate performance of the sweat is crucial for the accurate diagnosis of CF. Therefore, the CF Foundation requires the sweat testing conducted at accredited CF care centers adheres to the standards recommended by the Cystic Fibrosis Foundation committee comprising CF center directors.[15] Our study showed elevated chloride levels among CF patient CFTR mutation I1234V with a mean and standard deviation for both Gibson-Cooke method and WMSC of 99.22±8.34 and 96.37±11.83, respectively. Regarding to the second comment on sweat conductivity, it should not be used for diagnosing CF at the present time;[3] hence, we used WMCS for measuring conductivity instead of nanoduct system and both are screening tests. Being our CF cohort is small (41 patients with a median age of 12.25 years, ranging from 1.3-31 years), which did not make a cut-off value of sweat conductivity and the present study have shown in agreement as chosen by Lezana et al[7] study.

Atqah Abdul-Wahab
Ibrahim A. Janahi
Department of Pediatrics
Mohammed O. Abdel-Rahman
Department of Biochemistry
Hamad Medical Corporation
Doha, Qatar

References

1. Abdul-Wahab A, Janahi IA, Abdel-Rahman MO. Sweat chloride concentration in cystic fibrosis patients with cystic fibrosis trans-membrane conductance regulator 11234V mutation. ***Saudi Med J*** 2009; 30: 1101-1102.
2. van den Bergh FA, Martens A. Diagnosis of cystic fibrosis: simple genotyping to rule out the disease preferable to starting with the sweat test. ***Ned Tijdschr Geneeskd*** 2003; 147: 1001-1005.
3. Riedler J, Arrer E. Comparison of the classical Gibson-Cooke methods and the chloride-sensitive electrode in sweat testing for diagnosis of cystic fibrosis. ***Padiatr Padol*** 1991; 26: 173-175.
4. Hammond KB, Turcios NL, Gibson LE. Clinical evaluation of the macroduct sweat collection system and conductivity analyzer in the diagnosis of cystic fibrosis. ***J Pediatr*** 1994; 124: 255-260.
5. Mastella G, Di Cesare G, Borruso A, Menin L, Zanolla L. Reliability of sweat-testing by the Macroduct collection method combined with conductivity analysis in comparison with the classic Gibson and Cooke technique. ***Acta Paediatr*** 2000; 89: 933-937.
6. Seia M, Costantino L, Paracchini V, Porcaro L, Capasso P, Coviello D, et al. Borderline sweat test: utility and limits of genetic analysis for the diagnosis of cystic fibrosis. ***Clin Biochem*** 2009; 42: 611-616.
7. Lezana JL, Vargas MH, Karam-Bechara J, Aldana RS, Furuya ME. Sweat conductivity and chloride titration for cystic fibrosis diagnosis in 3834 subjects. ***J Cyst Fibros*** 2003; 2: 1-7.
8. Desax MC, Ammann RA, Hammer J, Schoeni MH, Barben J, Swiss Paediatric Respiratory Research Group. Nanoduct sweat testing for rapid diagnosis in newborns, infants, and children with cystic fibrosis. ***Eur J Pediatr*** 2008; 167: 299-304.
9. Grody WW. Cystic fibrosis: molecular diagnosis, population screening, and public policy. ***Arch Pathol Lab Med*** 1999; 123: 1041-1046.
10. Serre JL, Feingold J. Conditions and limitations of healthy carrier screening for the mutation responsible for cystic fibrosis. ***Rev Epidemiol Sante Publique*** 1993; 41: 353-362.
11. Abdul Wahab A, Al Thani G, Dawod ST, Kambouris M, Al Hamed M. Heterogeneity of the cystic fibrosis phenotype in a large kindred family in Qatar with cystic fibrosis mutation (I1234V). ***J Trop Pediatr*** 2001; 47: 110-112.
12. O'Sulivan BP, Freedman SD. Cystic Fibrosis. ***Lancet*** 2009; 373: 1891-1904.
13. Farrell PM, White TB, Cutting GR, Hazle L, Michael Knowles M, Marshall B, et al. Guidelines for diagnosis of cystic fibrosis in newborns through older adults: Cystic Fibrosis Foundation consensus report. ***J Pediatr*** 2008; 153: S4-S14.
14. Mishra A, Greaves R, Smith K, Carlin JB, Wootton A, Stirling R, et al. Diagnosis of cystic fibrosis by sweat testing: age-specific reference intervals. ***J Pediatr*** 2008; 153: 758-763.
15. LeGrys VA, Yankaskas JR, Quittell LM, Marshall BC, Mogayzel PJ Jr; Cystic Fibrosis Foundation. Diagnostic sweat testing: the Cystic Fibrosis Foundation guidelines. ***J Pediatr*** 2007; 151: 85-89.