Sweat chloride concentration in cystic fibrosis patients with cystic fibrosis trans-membrane conductance regulator I1234V mutation

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Cystic fibrosis (CF) is one of the most common inherited diseases in Caucasian people. It is caused by mutations in both the cystic fibrosis transmembrane conductance regulator (CFTR) gene that encodes a transmembrane glycoprotein.[1] One of the main consequences of mutations in the CFTR gene is a dysfunction of ion channels resulting in elevated sweat chloride concentrations, pancreatic insufficiency, and progressive lung disease.[2] Sweat testing is a general term referring to the quantitative, or qualitative analysis of sweat to determine the electrolyte concentration, conductivity, or osmolarity for confirmation of a CF diagnosis, and despite the development of genetic testing, analysis of sweat chloride concentration remains the laboratory gold standard for the diagnosis of CF.[3] The cut-off chloride value of 60 mEq/L is considered frankly abnormal. It is possible to make diagnosis of CF even with a chloride value <60 mEq/L in the presence of other signs.[3] Since the inception of the sweat test 50 years ago, the pad-absorption technique using quantitative pilocarpine iontophoresis test (QPIT) is probably the one most commonly used, and has been accepted as the standard for sweat testing.[4] Sweat conductivity represents a non-selective measurement of ions. A Wescor Sweat-Chek conductivity analyzer (Wescor Inc., Logan, Utah, USA) designed specifically for use with Wescor Macroduct sweat collector (WMSC [Wescor Inc., Logan, Utah, USA]), was approved by the Cystic Fibrosis Foundation as a screening method.[3] The first objective of this study was to define the pathologic range of chloride level in a sweat test in CF patients with CFTR I1234V mutation, common in a large kindred Arab tribe in the Gulf region,[5] utilizing the gold standard QPIT (Gibson-Cooke test), which has become available for the first time in our institution. The second objective was to compare the QPIT with the WMSC system for measuring chloride concentration and sweat conductivity.

Forty-one patients (1.3-31 years old), known to have CF with homozygous CFTR mutation I1234V were compared with 18 healthy volunteers (2 children, and 16 adult students and laboratory staff). Cystic fibrosis was diagnosed earlier on the basis of positive sweat results in duplicate using Hitachi 911 (Sysmed Lab Inc., Tokyo, Japan) ion-selective electrodes (chloride concentration >60 mmol/L), and a positive result on gene mutation analysis, plus clinical symptoms, and a positive family history of CF. Informed consent was obtained for all study subjects, and ethical clearance was obtained from the research committee of the Medical Research Centre, Hamad Medical Corporation, Doha, Qatar before enrolling participants in the study.

Each patient was tested by QPIT on the right forearm, and by the Macroduct sweat collection system, and chloride and sweat conductivity determination on the left forearm on the same day. All tests were carried out by experienced personnel by following the guidelines of the National Committee for Clinical Laboratory Standards at Hamad Medical Corporation, Qatar, from May 2007 to February 2008. The QPIT was performed with measurements of sweat weight, and chloride concentrations. Sweat specimens were collected concurrently from the right arm by first cleaning the skin with water, then applying a 2-inch-square salt-free gauze pad soaked with 0.5% pilocarpine solution, and covered with a 33-mm-diameter metal electrode. After a 4-minute pause, the skin was rinsed with water and dried thoroughly, reweighed gauze was then applied over the stimulated area, and covered with a Parafilm. After 30 minutes the Parafilm was lifted, the gauze removed with forceps, and then rapidly reweighed in an analytical balance before the electrolytes were eluted with water. A minimum sweat weight of 50 mg was required for analysis. Chloride concentrations were analyzed with a digital Jenway chloridometer (Jenway Ltd, Essex, UK), colorimetric titration method. On the left arm, sweat simulation was achieved by pilocarpine iontophoresis from disposable gel discs, 2.5 cm in diameter that were fitted into circular recessed stainless steel electrodes. Current was applied with a controlled rate-of-change to avoid patient discomfort, and collection was facilitated by a disposable plastic device that provided a shallow concave surface designed to cover precisely the circular skin area previously stimulated. A small amount of a water-soluble dye (10 μL) on the concave surface of the disk allowed easy visualization of the sweat collected. The amount of sweat was sufficient when the capillary was filled (approximately 30 minutes). Both sweat conductivity and chloride concentration was then measured using a Sweat-Chek conductivity analyzer, and a Sherwood colorimeter (Sherwood Scientific Ltd, Cambridge, UK)).

Descriptive statistics (mean, median, and standard deviation) were calculated and the associations between

chloride levels utilizing Gibson-Cooke test and WMSC, as well as sweat chloride level, and sweat conductivity, such as Pearson correlation coefficient (correlation of average).

Both the QPIT (Gibson-Cooke method), and the WMSC system method were successful in producing sufficient sweat in all 59 subjects. The mean sweat chloride level ± SD with QPIT method in CF patients with I1234V mutation was 99.2 ± 8.3 mmol/L and 15.4±6.7 mmol/L for the control group. The mean sweat chloride level ± SD with the Macroduct sweat collection system in CF patients with I1234V mutation and for the control group is shown in **Table 1**. As expected, both the chloride levels and conductivity values for CF patients with I1234V mutation were much higher than those observed in non-CF subjects (**Table 1**). There was excellent correlation between the results of sweat chloride concentrations by both the QPIT and WMSC method, and sweat conductivity.

In both procedures, the total chloride-ion-concentration in the sweat of healthy subjects, and patients with CF differed significantly, indicating that both the original QPIT and the WMSC system are highly reliable procedures, being diagnostically equivalent as suggested in an earlier report.[6]

Table 1 - Comparison between CF group and control group.

Sweat chloride levels method	CF group (n=41)	Control group (n=18)	*P*-value
Gibson and Cooke (mmol/L)			<0.001
Mean ± SD	99.2 ± 8.3	15.4 ± 6.7	
Median	100.70	14.9	
Range	78.3-118.2	5.5-27.9	
Wescor Macroduct			<0.001
Mean ± SD	96.4 ± 11.8	15.8 ± 6.0	
Median	97.00	16.00	
Range	66-120	7-32	
Sweat conductivity (equivalent NaCl in mmol/L)			<0.001
Mean ± SD	117.05 ± 10.09	36.83 ± 7.49	
Median	117.00	36.50	
Range	87-138	25-51	

CF - cystic fibrosis, NaCl - sodium chloride, SD - standard deviation

Conductivity represents a non-selective measurements of total ions present in the sweat sample, mainly chloride,$^-$ sodium,$^+$ and potassium,$^+$ but also lower amounts of bicarbonate, lactate, calcium, magnesium, sulfate, and phosphate. Lezana et al,[7] found 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. We consider that both the original QPIT and the WMSC system are highly reliable procedures for measuring sweat chloride concentrations for CF diagnosis, however, a positive sweat conductivity should always be confirmed by another sweat test measuring chloride concentration, or by gene mutation analysis.

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