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

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Sweat test with Gibson Cooke (GC) method is the diagnostic gold standard for cystic fibrosis (CF). Recently, alternative methods have been introduced to simplify both the collection and analysis of sweat samples.

Our aim was to compare sweat chloride values obtained by GC method with other sweat test methods in patients diagnosed with CF and whose CF diagnosis had been ruled out. We wanted to determine if the other sweat test methods could reliably identify patients with CF and differentiate them from healthy subjects.

Chloride concentration was measured with GC method, chloride meter and sweat test analysis system; also conductivity was determined with sweat test analysis system.

Forty eight patients with CF and 82 patients without CF underwent the sweat test, showing median sweat chloride values 98.9 mEq/L with GC method, 101 mmol/L with chloride meter, 87.8 mmol/L with sweat test analysis system. In non-CF group, median sweat chloride values were 16.8 mEq/L with GC method, 10.5 mmol/L with chloride meter, and 15.6 mmol/L with sweat test analysis system. Median conductivity value was 107.3 mmol/L in CF group and 32.1 mmol/L in non CF group. There was a strong positive correlation between GC method and the other sweat test methods with a statistical significance (r=0.85) in all subjects.

Sweat chloride concentration and conductivity by other sweat test methods highly correlate with the GC method. We think that the other sweat test equipments can be used as reliably as the classic GC method to diagnose or exclude CF.

Key words: cystic fibrosis, sweat chloride, conductivity, sweat test, diagnosis.

Cystic fibrosis (CF) is the most common fatal inherited disease that affects both children and adults, and is caused by a defect in the cystic fibrosis transmembrane conductance regulator (CFTR) protein[1]. CF is diagnosed based on ≥1 of the typical clinical features, a positive neonatal CF screening result, or a history of CF in a sibling and confirmation of CFTR protein dysfunction[2-4]. CFTR protein functional abnormalities result in impaired electrolyte transport in secretory and absorptive epithelia, including the reabsorptive duct of the sweat glands, which causes elevated salt loss via the sweat glands[5].

Currently, the sweat test is the most widely used and most conclusive biochemical method for diagnosing CF[5,6]. Measurement of the sweat chloride concentration via the quantitative pilocarpine iontophoresis test (QPIT), as

described by Gibson and Cooke, is considered to be the most accurate diagnostic method for CF and is widely accepted as the gold standard for sweat testing[7-9]. In general, the diagnosis of CF can be made in a patient with clinical features of the disease if the concentration of chloride in sweat is greater than 60 mmol/L or if it is in the intermediate range (30-59 mmol/L) and two disease-causing CFTR mutations are identified[3,4]. However, this diagnostic method involves multiple steps for collection and analysis of a sweat sample, is time consuming, and is associated with the risk of volumetric, gravimetric, condensate, and evaporation errors, especially in laboratories that do not routinely perform sweat testing[9-11].

Alternative methods have been introduced to simplify both the collection and analysis of sweat samples[12-14]. The measurement of sweat conductivity is a method that is increasing in popularity, because it is easier to perform and requires a smaller sample volume (minimum 6 μL) than QPIT (min 150 mg)[15-18]. Although some studies have shown that the conductivity method correlates well with the chloride concentration, it is currently not accepted by the National Committee for Clinical Laboratory Standards (NCCLS) as a definitive diagnostic tool and the Cystic Fibrosis Foundation (CFF) designates it as a 'screening' method[8,9,19]. According to CFF, individuals with sweat conductivity ≥50 mmol/L should be referred for QPIT quantitative sweat chloride testing[4].

The objective of the present study was to compare the sweat chloride concentration obtained using the GC method to that obtained using a chloride meter (Sherwood M926S Chloride Analyzer®) and a sweat test analysis system (CFΔ Collection System®) in patients diagnosed with CF and in individuals in whom CF diagnosis was ruled out in order to determine if the chloride meter and sweat test analysis system could reliably differentiate patients with CF and healthy individuals.

Material and Methods

Subjects

The study included patients known to have CF (CF group) and individuals that were referred to our laboratory due to suspicion of CF that did not have the disease (non-CF group). In the CF group, patients known as CF with clinical findings and laboratory evidence of CFTR dysfunction in the form of elevated sweat chloride concentrations on at least two occasions and/or presence of two CF mutations were included. Forty eight of the patients had CF while 82 did not but had been referred to the pediatric chest disease department because of other clinical findings. Informed consent was obtained from all subjects or their parents, and the Hacettepe University Ethics Committee approved the study protocol. CF was diagnosed based on at least two positive Gibson Cooke sweat test results, or a positive CFTR gene mutation analysis result and clinical findings of CF.

On the same day and in the same patient, the sweat test analysis system (CFΔ Collection System®) was used to measure sweat conductivity and the chloride concentration; the chloride meter (Sherwood M926S Chloride Analyzer®) was used to measure the chloride concentration to compare with the GC method. The tests were performed on the left (CFΔ Collection System®, Sherwood M926S Chloride Analyzer) and right forearms (GC method), respectively. All tests were performed between June 2013 and August 2013 at Hacettepe University Children's Hospital, Division of Pediatric Pulmonology Ankara, Turkey, by three trained technicians.

Based on the available data on sweat chloride test results, the following sweat chloride reference ranges were used: CF is very unlikely in individuals with Cl^- <30 mmol/L; Cl^- =30-59 mmol/L is intermediate; and Cl^- ≥60 mmol/L is indicative of CF (3). The intermediate group comprised six CF patients.

The sweat chloride concentration based on the Gibson Cooke method

The sweat test was performed in three stages: stimulation of sweating with iontophoresis, collection of sweat sample and analysis. In the first stage (iontophoretic stimulation), the forearm skin was cleaned with distillated water and dried. A gauze bandage (2x2 cm) dampened with pilocarpine solution was placed on the forearm near the wrist and a positive electrode was placed on it and strapped. The electrode was attached to the positive pole of the iontophoresis instrument (Model 4013 Union®). The second gauze bandage (2x2 cm) dampened with 0.02 N K_2SO_4 solution

was placed on the forearm above the elbow and a negative electrode was placed on it and strapped. This electrode was attached to the negative pole of the instrument. Then a current of 2.5-3 mA was applied during a five-minute period.

The second stage was sweat collection. A weighed 4x4 cm filter paper was placed near the wrist and closed with parafilm. After waiting 30 minutes for collecting sweat, the filter paper was taken and weighed again.

The third stage was analysis. Filter paper with at least 100 mg sweat was washed with 3 ml distilled water (if the collected amount of sweat was >150 mg, it was washed with 5 ml distilled water). 1 ml solution was taken from this bath and placed in a clean tube. Two drops of 2N HNO_3 and 3 drops of S-diphenylcarbazone solution (0.1% g/v) put in a tube and mixed. The mixture in the tube was titrated with 0.005 N mercury nitrate solution till a pink-purple color formed.

Sweat chloride concentration was calculated with the following equation:

Sweat chloride concentration (mEq/L) = (water amount added (ml) + weight of the sweat) x vol. $Hg(NO_3)_2$ x N x 1000

weight of the sweat

Vol. $Hg(NO_3)_2$: volume of mercury nitrate used in the titration;

N: normality of mercury nitrate used in the titration.

Sweat test analysis system

The CF Δ Collection System® sweat test analysis system (UCF 2010 Iontophoresis Unit and UCF 2011 Sweat Analysis Unit) is used to analyze the conductivity and chloride concentration of sweat via conductivity measurement by a coulometric end point software method.

In this method, there are 3 stages: obtaining sweat sample by iontophoretic stimulation of sweat glands, collection of sweat sample by a collector, which includes a microcapillary tube and analyzing phase.

At first stage, two electrodes carrying pilocarpine nitrate containing gel discs were placed over the forearm after cleaning the skin with deionized water and letting the skin dry. A maximum of 1.5 mA current was applied on these electrodes during 5-7.5 minutes period.

In the sweat-collecting stage, after cleaning and drying the skin, a CFΔ collector was placed over the skin where the positive electrode was located. The CFΔ collector is a disposable, concave, plastic disc attached to a spiral plastic tube inside and sweat travels through this plastic tube and is captured; thus, the risks of dead space and evaporation disappear.

In 30 minutes, 50-60 microliters of sweat can be collected, and this amount is adequate for analyzing chloride concentration with both Sherwood chloride meter 926 S analyser and the CFΔ Collection System analyser of the same sample.

This method contains the measurement of number of electrons flowing through sweat sample by applying potential difference on two electrodes in micro volumed and constant temperature controlled measurement cell. It is possible to measure the conductivity and chloride concentration of sweat (4.1-6 microliters sweat sample) via coulometric end point software method with this instrument. Measurement is determined as mmol/L, and this unit represents the molar concentration of sweat chloride value and equivalent sodium chloride value for the same sweat sample, at the same temperature[20].

Analysis of sweat chloride via titration using a chloride meter

The chloride concentration of a sweat sample can be determined via titration using a chloride meter. The Sherwood M926S is a direct-reading digital chloride meter. Sweat samples were collected into CFΔ collector coils as previously mentioned above. The selectable sample volume is 100 μL or 20 μL and the results are displayed on a digital readout. Sherwood M926S is used to measure chloride ions; like the classic method, the Sherwood M926S relies on the chemical formation of the very insoluble salt, silver chloride. The Sherwood M926S automatically titrates chloride ions by passing a known constant current between two silver electrodes that provide a constant generation of silver ions. During the titration period the digital readout is updated approximately every 0.3 seconds. When all the chloride has been precipitated as silver chloride, free silver ions begin to appear and the solution conductivity

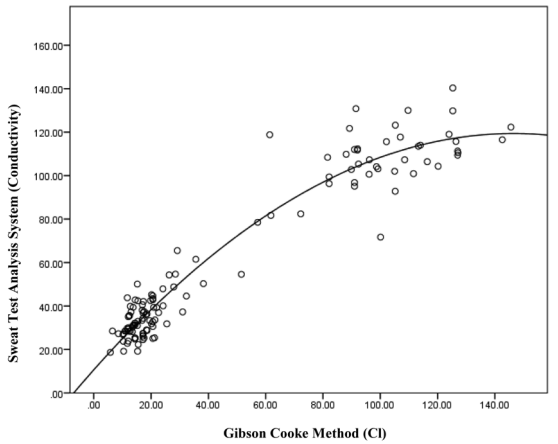

Fig. 1. Scatter graph of chloride concentration with Gibson Cooke method and conductivity with sweat test analysis system

changes. This change is detected by the sensing electrodes, and the readout stops and displays the results directly as mmol/L[21].

The coefficient of variation for sweat test analysis system, chloride meter and GC method ranges from 0.003 to 0.2; 0.06 to 0.24 and 0.09 to 0.1 respectively for each method[20,21].

Statistical analysis

statistical analysis was performed using SPSS version 20 for Windows. This cross-sectional diagnostic test study included a population of patients known to have CF and not to have CF. Because the conductivity and chloride concentration values were not normally distributed, a non-parametric approach was used for data evaluation. The relationship between conductivity and the chloride concentration was examined via Spearman's correlation test and the level of statistical significance was set at p 0.05. A Bland and Altman plot, a statistical method to compare two clinical measurement techniques, was utilized to assess agreement between Sherwood M926S Chloride Analyzer®, CFΔ Collection System® and Gibson Cooke method.

Results

Sweat tests were performed in 140 subjects aged between 7 months and 31 years; of these, 130 had a sweat rate (7.1%) that was adequate for assessment. CF patients (50% females) had a median age of 9.5 years ranging from 7 months to 31 years. Non-CF patients (48.7% females) had a median age of 6.5 years ranging from 7 months to 18 years. Table I shows the results obtained with the GC method, sweat test analysis system and chloride meter in patients grouped according to the presence of CF disease. As expected, conductivity values for CF group were much higher than chloride concentration.

There was a strong positive correlation between the GC method and the two other sweat test methods ($p \leq 0.001$) in all subjects (Table II). In the CF group there was a moderate positive ($p \leq 0.001$) correlation between the two measurements ($r=0.54$, $r=0.58$). In the non-CF group the correlation was positive, moderate, and statistically significant ($p \leq 0.001$, $r=0.52$). There was a non-linear relationship between chloride concentration (GC method) and conductivity (sweat test analysis system)

Table I. Sweat Chloride and Conductivity Values in Patients with CF (n=48) and non-CF (n=82) Patients

	Sweat Test Analysis	System	Chloride meter	Gibson Cooke Method
	Chloride (mmol/L)	Conductivity (mmol/L)	Chloride (mmol/L)	Chloride (mEq/L)
Patients with CF				
Median	87.8	107.3	101	98.9
Mean±SD	85±18.2	104.4±18.9	94.9±23.7	98±24.8
Min-Max	33.1-119.5	50.3-140.3	32-156	35.6-145.6
Non-CF Patients				
Median	15.6	32.1	10.5	16.8
Mean±SD	17.5±8.4	34±8.8	11.5±4.9	16.8±5.3
Min-Max	2.7-47.7	18.7-65.5	4-30	5.7-32.3

CF: Cystic Fibrosis SD: Standard Deviation Min: Minimum Max: Maximum

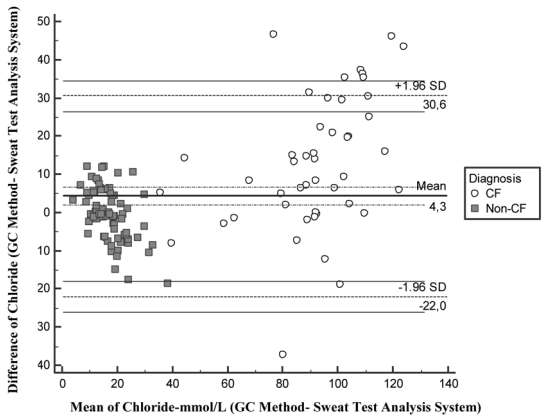

Fig. 2. Chloride concentration agreement between Gibson Cooke method and sweat test analysis system

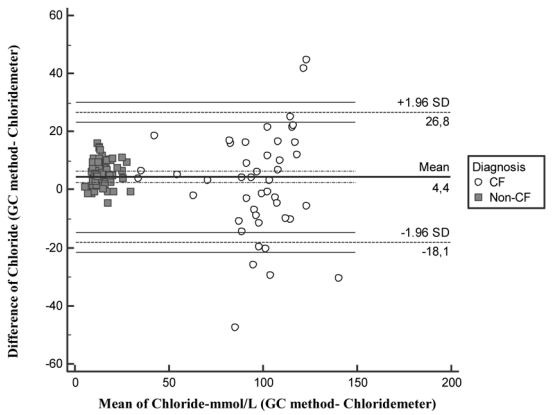

Fig. 3. Chloride concentration agreement between Gibson Cooke Method and chloride meter

(Fig. 1). Correlation analysis between the two diagnostic methods yielded r=0.85 (p≤0.001).

There was reasonable agreement between the different sweat chloride measurement and GC method in CF and non CF subjects: The 95% limits of agreement for chloride (GC method-Sweat Test Analysis System) are from -22 to 30.6 mmol/L and GC method-Chloride meter are from -18.1 to 26.8 mmol/L (Figs. 2 and 3). The agreement is less for higher chloride values.

Discussion

Diagnosing CF is not always simple, but measurement of the sweat chloride concentration remains the gold standard. Quantitative analysis of the sweat chloride concentration is widely accepted as the most discriminatory test for diagnosing CF[22].

New alternative and time efficient methods have been introduced to simplify both the collection and analysis of sweat samples. The present study compared the GC method to a chloride meter (Sherwood M926S Chloride Analyzer®) and sweat test analysis system (CFΔ Collection System®). The primary finding of the present study is that chloride meter testing and the sweat test analysis system both had high capacity to differentiate between those with and without CF. In addition, the sweat test analysis system (CFΔ Collection System®) which measures both the chloride

Table II. Correlation of Values Between Gibson Cooke Method and the Other Sweat Test Methods in all Subjects

Gibson Cooke Method		Sweat Test Analysis Chloride (mmol/L)	System Conductivity (mmol/L)	Chloride meter Chloride (mmol/L)
All patients	r	0.85	0.85	0.85
	p	p≤0.001	p≤0.001	p≤0.001
Patients with CF	r	0.58	0.58	0.54
n=48	p	p≤0.001	p≤0.001	p≤0.001
Non-CF patients	r	0.52	0.52	0.52
n=82	p	p≤0.001	p≤0.001	p≤0.001
Negative n=81	r	0.49	0.49	0.49
<30 mEq/L	p	p≤0.001	p≤0.001	p≤0.001
Intermediate n=6	r	0.82	0.82	0.82
30-59 mEq/L	p	0.04	0.04	p≤0.001
Positive n=43	r	0.41	0.41	0.35
≥60 mEq/L	p	0.006	0.006	0.02

concentration and conductivity together, might be an alternative method.

The sweat conductivity method is increasing in use for the diagnosis of CF at many laboratories. Whereas conductivity is presently considered a screening test only, some studies have proposed its use for diagnosis[18-19]. Sweat conductivity includes the measurement of other unmeasured charged ions in sweat, including lactate, bicarbonate, sodium, potassium and chloride; therefore, conductivity is approximately 15 mmol/L higher than sweat chloride alone[22]. In the present study conductivity values were higher than the other tests according to this.

In this study, the results revealed that chloride meter (Sherwood M926S Chloride Analyzer®) testing and the sweat test analysis system (CFΔ Collection System®) were nearly equivalent to the GC method and showed strong agreement between all the methods studied. There was a strong correlation between GC method and the other sweat test methods in all subjects (r=0.85). There was also a moderate positive correlation in the patients in the CF and non-CF groups. Lezana et al.[11] compared conductivity and classic sweat test methods in 3834 patients (294 with CF) and reported that there was a strong correlation between the methods for confirming and ruling out the diagnosis of CF (r=0.6). Hammond et al.[15] described the relationship between conductivity values and the chloride concentration in sweat samples obtained from 471 subjects and 43 CF subjects; they observed a high correlation coefficient (r=0.97) in all CF patients.

Cinel et al.[23] analyzed 59 CF patients and 69 non-CF patients and reported that there was a strong positive correlation between conductivity and the chloride concentration (r=0.88) in all subjects. In their CF group there was a weak positive correlation (r=0.33) and in the non-CF group there was a moderate positive correlation (r=0.67). Rose et al.[24] also reported that conductivity was strongly correlated with the chloride concentration (r=0.93).

Mastella et al.[18] reported that conductivity measurement had good sensitivity and specificity; all patients identified via classical QPIT were considered positive based on conductivity. They also noted a strong correlation between both techniques based on administration of the tests to 287 individuals: among the 184 patients without CF the mean chloride concentration was 16.3 mmol/L (range 4 to 60 mmol/L) and mean conductivity was 39.8 mmol/L (range from 19 to 87 mmol/L), whereas among the 103 CF patients the mean chloride concentration was 95.7 mmol/L (range 32 to 121 mmol/L) and mean conductivity was 112 mmol/L (range 45 to 173 mmol/L). The conductivity test had a similar ability to differentiate those with and without CF[18].

Mattar et al.[25] observed that the conductivity test had high sensitivity and specificity in 52 CF patients and 50 non-CF patients, and that there was good concordance between the tests.

GC method involves multiple steps for collection and analysis of a sweat sample; it is also time consuming for analyzing the results and needs experienced technicians. These new alternative methods in this study allow to collect 50-60 microliters of sweat in 30 minutes, and this amount is adequate for analyzing chloride concentration with both sweat test analysis system and chloride meter.

The sweat test analysis system (CFΔ Collection System®) used in the present study was observed to be less complicated and was able to analyze sweat electrolytes in smaller samples than other sweat test methods. This system has the advantage of collecting the sweat directly and analyzing both the chloride concentration and conductivity simultaneously via coulometric end point software method. This method also allows visualization of how much sweat is collected at any time of the procedure.

The chloride meter (Sherwood M926S Chloride Analyzer®) used in the present study was observed as a reliable method of measuring the chloride concentration via titration, using as little as 20 μL of sweat. This is also a direct-reading digital chloride meter and it is time efficient for analyzing the results.

The limitation of this study is the overall sufficient sweat rate of 7.1% is above the recommended rate of <5% reported by Le Grys et al[6]. Also we could not give the results for coefficient of variation in this trial. We have the general results for coefficent of variation for each test.

Conclusion

In conclusion, the CFΔ Collection System® and Sherwood M926S Chloride Analyzer® can be

reliably used in conjunction with other tests for the diagnosis of CF.

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