Sweat Chloride Test

Introduction

Cystic Fibrosis is one of the most common, life limiting, inherited, multi - organ disorders of childhood. It was a well known fact that the children who suffered from Cystic Fibrosis (CF), tasted salty when kissed. As per an old European saying, "'Woe to that child which when kissed on the forehead tastes salty. They are bewitched and soon will die'. The disease was soon found to be correlated with excess of sodium & hence chloride the sweat.

Sweat chloride testing emerged as one of the gold standard tests for diagnosing CF. It is a reliable test for the diagnosis of CF in approximately 98% of patients with CF.

But why are we testing for sweat chloride in an era of Genomics?

Great advancements have been made in the field of Genomics. This has led to a better understanding of the molecular processes causing CF. We are now empowered with a new set of advanced and accurate diagnostic modality.

CF has been found to be associated with abnormality in a gene called as the CFTR gene or the cystic fibrosis trans membrane regulator gene.

The CFTR gene encodes a trans-membrane glycoprotein. This acts as an electrolyte transporter at the apical membrane of epithelial cells. Since the discovery of the gene, over 1200 disease-associated mutations have been identified.

However, in view if the large number of mutations available, routine screening tests are not able to detect all CFTR gene mutations, and a negative screening test does

not ensure a normal CFTR genotype.

Genotyping has also revealed that some CF mutations are associated with milder disease phenotypes and normal or borderline abnormal concentration of sweat electrolytes. So, the utility if these tests is largely for carrier identification, prenatal diagnosis in at-risk pregnancies and newborn screening programs for CF. Cost too, is a limiting factor. However, as there are > 1000 mutations & 200 polymorphisms, many without recognised effects on CFTR, the molecular diagnosis can be troublesome and hence the renewed interest in the sweat test.

The Sweat Test

Sweat testing is a general term referring to the quantitative or qualitative analysis of sweat to determine electrolyte concentration, conductivity, or osmolality for the diagnosis of CF.

The principle indications for performing a sweat test include:

1. Positive newborn screening for CF (elevated IRT followed by CFTR mutation analysis)
2. Clinical signs suggestive of CF
3. or a family history of CF

Other Indications for Sweat Testing

It is indicated in all suspected cases of CF. Likely phenotypes of CF are:

1. **Chronic sinopulmonary disease manifested by**

a. Persistent colonization / infection with typical CF pathogens including Staphylococcus aureus, nontypeable Haemophilus influenzae, mucoid and

nonmucoid Pseudomonas aeruginosa , and Burkholderia cepacia

b. Chronic cough and sputum production

c. Persistent chest radiograph abnormalities (e.g. bronchiectasis, atelectasis, infiltrates, hyperinflation)

d. Airway obstruction which can manifest by wheezing and air trapping

e. Nasal polyps; radiographic or computed tomographic abnormalities of the paranasal sinuses

f. Digital clubbing

2. **Gastrointestinal and nutritional abnormalities including**

a. Intestinal: meconium ileus, distal intestinal obstruction syndrome, rectal prolapse

b. Pancreatic: pancreatic insuffi ciency, recurrent pancreatitis

c. Hepatic: chronic hepatic disease manifested by clinical or histologic evidence of focal biliary cirrhosis or multilobular cirrhosis

d. Nutritional: failure to thrive (protein-calorie malnutrition), hypoproteinemia and edema, complications secondary to fat-soluble vitamin deficiency

3. **Salt loss syndromes**: acute salt depletion, chronic metabolic alkalosis

4. **Male urogenital abnormalities** resulting in obstructive azoospermia (CBAVD)

Pitfalls of the Sweat Analysis

Most errors relating to sweat tests are caused by not following standard protocols, inadequate sweat collection, technical errors and, occasionally, misinterpretation of the results. The technical aspects of performing a sweat test are demanding and these errors occur more often in institutions doing relatively few tests, usually not in accordance with published guidelines.

False Positive Sweat Test

Approximately 98% of patients with CF have sweat chloride concentrations greater than 60 mmol/L. There are a variety of well described, although rare conditions, which are associated with elevation of sweat electrolytes. They are:

a. atopic eczema
b. untreated Addison's disease
c. ectodermal dysplasia,
d. Some types of glycogen storage diseases, and
e. untreated hypothyroidism
f. Sweat electrolytes measured within the first 24 hours after birth may also be transiently elevated. Up to 25% of normal newborns show a sweat sodium concentration greater than 65 mmol/L on day 1 but this rapidly declines on the second day after birth.
g. Technical errors such as evaporation and contamination, except for the misdilution of sample

False Negative Sweat Test

The most important pathophysiological cause of a false negative sweat test is oedema. Oedema is commonly seen in infants with hypoproteinaemia, which can be secondary to pancreatic exocrine insufficiency, before diagnosis and
treatment with replacement pancreatic enzymes. The use of mineralocorticoids can also decrease sweat electrolyte concentrations.

From a technical point of view, the rate of sweating is important in achieving

accurate results as the sweat electrolyte concentration is related to sweat rate. At low sweat rates, sweat electrolyte concentration decreases and the opportunity for sample evaporation is increased. The average sweat rate should exceed 1g/m2 /min.

Insufficient samples can be due to several factors such as age, skin condition, hydration status and collection system.

At what age can we do Sweat test ?

As a general guideline, sweat tests can reliably be performed after 2 weeks of age in infants greater than 3 kg and occasionally the sweat test can be attempted in term infants after 7 days of age

Sweat Stimulation Site

- Flexor surface of the forearm
- Other sites - upper arm, thigh & calf

Diagnostic Dilemma

One faces the following difficulties in interpretation:

- During infancy, sweat chloride $\geq$ 40 mmol/L has a low probability of being a true normal, & a sweat chloride level between 40-59 mmol/L is likely to be diagnostic for CF
- Therefore, Infants with borderline sweat chloride values need follow-up, careful observation, repeat sweat testing & extended CFTR mutation analysis

Procedure of sweat collection

- The flexor aspect of forearm is cleaned with distilled water and dried with clean swabs.

- Two pieces of filter (Whatman filter paper no 42 size 2.5 × 2.5 cm) soaked in 1% pilocarpine solution are placed under positive electrode and the electrode is secured over the flexor aspect of the forearm with the help of roller guaze.
- Similarly flexor aspect of arm in the same limb is cleaned and 5 filter papers soaked in plain water are placed under negative electrode and it is secured on the arm with roller guaze.
- The completion of circuit is checked from reading of multimeter.
- The iontophoresis procedure is continued for 5-7 minutes.
- The value of maximum current is recorded from multimeter.
- If the observed current value was less than 1.5 mA, iontophoresis is repeated.
- After 5-7 minutes of iontophoresis, electrodes are removed and skin under the positive electrode was cleaned with distilled water and dried with swabs.
- Filter paper from the pre-weighed bottle (bottle weighed with filter paper) is taken out with the help of forceps and placed over the cleaned skin.
- The filter paper was secured firmly to skin with the help of a cling film. After 30 minutes filter paper is removed and put back into the bottle.
- The bottle along with filter paper containing sweat is weighed on an electronic weighing scale with an accuracy of 0.1 mg.
- The weight of the collected sweat is calculated by the difference in weight (before and after sweat collection). The minimum sweat weight that could be used for titration is 100 mg. If the sweat weight is less than 100 mg, iontophoresis is repeated.

- The sweat in filter paper is eluted in 8 mL distilled water over– night (12-16 hours).

Sweat chloride estimation

Sweat chloride is estimated by Schales and Schales method. It includes titration on 2 milliliters of eluted solution with 0.002N mercuric nitrate using diphenyl carbazone as indicator. The amount of mercuric nitrate required for titration is recorded in milliliters. Titration is repeated with 0.01N saline. The chloride is calculated by following equation N1 V1 = N2 V2.

Conclusion

The genomic era for CF started on a very promising note. It has led to a better understanding of the many functions of CFTR and has solved many of the diagnostic dilemmas confronting clinicians. At the same time the complexity of the diagnosis has increased with the recognition of milder phenotypes and patients with no clinical manifestation detected by screening programs waiting to see if their mutations will be clinically relevant. At the other end of the spectrum, there are patients with CF phenotypes in the absence of CFTR mutations.

Mutation analysis informs us about the gene, but not the gene product (mRNA or protein) or its function. Mutation analysis is often least helpful when the diagnosis is in greatest doubt.

Diagnosis that remains unclear after sweat testing and mutation analysis may be confirmed by other tests of CFTR function such as NPD. However, NPD can be inconvenient to perform on infants and its use as a diagnostic tool requires

further evaluation.

Hence diagnosis of CF needs a thorough clinical history and examination, mutation analysis, and sweat test to demonstrate the chloride transport abnormality.