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Short Communication

The role of sweat testing in cystic fibrosis

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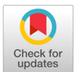

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Cystic fibrosis (CF) is an autosomal recessive genetic disease caused by mutations in the long arm of chromosome 7. The estimated prevalence of mutations in the CFTR gene is 1:25 in a Caucasian population. Very little data is present when it comes to Indians. lt is believed that the prevalence of Cystic Fibrosis disease in Asians is I in 90000 live births. The disease affects most of the internal organs but most of the morbidity and 90-95 % of mortality result from chronic pulmonary infections.

The gene responsible has been localized to 250,000 base pairs of genomic DNA on chromosome 7q (the long arm). It encodes a membrane-associated protein called the cystic fibrosis transmembrane regulator (CFTR). The most common gene mutation, $_{D}F_{508}$, leads to absence of a phenylalanine residue at position 508 on the CFTR protein and is found on about 70% of CF alleles; less common mutations account for the remaining 30%. Although the exact function of CFTR is unknown, it appears to be part of a cAMP-regulated Cl channel and appears to regulate Cl and Na transport across epithelial membranes. Heterozygotes may show abnormalities of epithelial transport but are clinically unaffected.

CF does not follow the same pattern in all patients but affects different people in different ways and to varying degrees. The basic problem, however, is the same—an abnormality in the glands that produce or secrete sweat and mucus. Nearly all exocrine glands are affected in varying distribution and degree of severity. Involved glands are of 3 types: those that become obstructed by viscid or solid eosinophilic material in the lumen (pancreas, intestinal glands, intrahepatic bile ducts, gallbladder, submaxillary glands); those that are histologically abnormal and produce an excess of secretions (tracheobronchial and Brunner's glands); and those that are histologically normal but secrete excessive Na and Cl (sweat, parotid, and small salivary glands). Fifty percent of patients present with pulmonary manifestations, usually chronic cough and wheezing associated with recurrent or chronic pulmonary infections. Pancreatic insufficiency is clinically apparent in 85 to 90% of patients, usually presents early in life, and may be progressive. Manifestations include the frequent passage of bulky, foul-smelling, oily stools; abdominal protuberance; and poor growth pattern. The diagnosis of CF is suggested by its characteristic clinical and laboratory features and confirmed by a sweat test.

1. The Sweat Test

At a Cystic Fibrosis Foundation GAP Conference on the sweat test, it was stated, "the diagnosis of cystic fibrosis must include a carefully performed quantitative pilocarpine iontophoretic sweat test which is interpreted by an experienced clinician". The purpose of this article is to describe the Quantitative Pilocarpine lontophoresis Sweat

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testing apparatus and bring out some of the points to be kept in mind while performing the sweat test.

By quantitative pilocarpine iontophoretic sweat test, it is meant that sweating is induced by the iontophoresis of pilocarpine; that the sweat produced is collected either upon a gauze square, filter paper or a collection device, and that its weight is determined by measurement with an analytic balance. The sweat test comprises of 3 stages, sweat stimulation, sweat collection and sweat analysis. Stimulation is ideally done by pilocarpine iontophoresis using stimulators based on the Gibson –Cooke method or the Wescor Macroduct® System. These are battery-operated apparatus connected to stainless steel electrodes. On the electrodes are placed either gauze / Whatmans Filter paper No. 42 pads or Pilogel® discs. Both the pads and pilogel discs contain an aqueous solution of Pilocarpine Nitrate 0.2-0.5 %. The electrodes are strapped to the forearm after cleaning the flexor surface with spirit and water and allow to dry. Stimulation is carried out for 5 minutes with a current of 5 mA. At the end of 5 minutes the stimulation is stopped and the electrodes removed. The surface is blotted dry and the collection device is strapped on. The collection of sweat can be done using preweighed gauze pads or Whatman's No.42 filter paper. Alternatively a patented device like the Wescor collection device could be used. The collection should not exceed 30 minutes and not be for less than 20 minutes. Care should be taken to see that the sweat does not evaporate from the pads during this time. At the end of the collection period the pads are weighed again and the weight of the sweat calculated. The collected sweat is then eluted from the pad using type I water as the eluent. If the weight of the sweat is greater than 100 mg, 10 ml of water is used. For sweat between 75-100 mg 5 ml water is used. If the weight is less than 75 mg the quantity is insufficient. The pads are allowed to stand in the water for 20 minutes. Swirl flask and decant the water into a labeled 50 mL centrifuge tube. Cover tube tightly. Add another 10 mL of Type I H_2O to the flask with the pad (or 5 mL as the case may be above) and stopper flask. Allow flask to stand at room temperature for a second 20-minute period. Swirl flask and decant into the same 50 mL centrifuge tube used for the first part of the wash. Cover tube tightly and invert tube several times to mix. 4-ml Aliquots of this diluted sweat solution will be used to assay for Cl-concentration. If the Wescor collection device is used there is no need for dilution and the sweat collected is directly analyzed by conductivity or by using an ISE.

The determination of chloride from the pads is usually done using a chloridometer. The reading (A) from the instrument is converted into mmol/l of chloride for the sample using the following equation

$(A)\left(\frac{5}{2004}\right)\left(\frac{20{,}000+sweat\ weight[mg]}{sweat\ weight[mg]}\right)=$ sweat Cl(mmol/L)

If the weight of the sweat is between 75 to 100 mgs use 10,000 in the numerator instead of 20,000. If the Wescor Swet-Chek" Conductivity analyzer is used it is preferred to use a sample collected with a Wescor collection device. This method gives the result as equivalents of NaCI in mmol/L.

2. Quality Control

Collecting in duplicate from two different sites ensures quality. For chloride values less than or equal to 60 mmol/L, the two sites should agree within 10 mmol/L; for values above 60 mmol/L, the two sites should agree within 15 mmol/L.

Values greater than 160 mmol/L are not physiologically possible and should not be reported. The chloridometer /conductivity meter should also be periodically checked by using third party control solutions and calibrated if required.

3. Interpretation and Reference Intervals

Normal sweat chloride levels in healthy individuals are below 40 mmol/L. In the presence of clinical symptoms such as recurrent respiratory disease or malabsorption, a family history of cystic fibrosis (CF), or a positive newborn screen, the findings of a sweat chloride above 60 mmol/L (for children) is consistent with the diagnosis of CF. Borderline is 40-60 mmol/L and always warrants repetition of the test. A negative test should be repeated if the clinical picture is suggestive of CF.[1]

4. Discussion

The sweat test remains the gold standard for confirming the diagnosis of cystic fibrosis.[2] Experienced laboratory personnel in centres who carry out at least 30 sweat tests per annum should always perform the test.

In term infants, sweat electrolytes can be raised in the first 7 days after birth, especially in the first 48 hours of life.[3] It is more routine to undertake sweat testing of infants after 2 weeks of age as long as their weight is greater than 3 kg. They remain normally hydrated and in the absence of significant systemic illness. (Guidelines for the performance of the sweat test; Multi-Disciplinary working group, 2002) Sweat testing should be delayed in subjects who are either dehydrated, underweight (infants), systemically unwell, peripheral oedema or systemic steroids administration.[2,4] It is still unclear as to how antibiotic treatment can affect the results due to paucity of data.[5]

For sweat collection, the preferred site is the lower portion of the flexor aspect of forearm, as that area has a high density of sweat glands for optimum sweat yield. If the entire arm is too small, attach the collector on the inner thigh, care is taken to see that the collection is not contaminated by urine in infants. The selected site must be free of breaks, fissures and sign of inflammation. This is to rule out the possibility of contamination of the

sweat by serous exudates. The process of iontophoretic stimulation is a safe procedure if done correctly. The patients will have to have the procedure explained to them carefully, and should be made aware that there is a very small risk of complication. The most common one is some mild reddening of the skin. Burns to the skin occur very infrequently & this risk can be minimized by careful attention to technique. The following are the most common errors that occur in sweat testing. The use of an alternating current (AC) powered iontophoresis unit rather than a battery powered unit, sweat stimulation (iontophoresis) for longer than 5 minutes, touching filter paper or gauze with bare fingers, sweat collection for longer than 30 minutes, pooling of samples from two extremities, analysis of less than 75 mg of sweat, lack of analysis of controls and a quality assurance protocol. Some mutations such as RI 17H, 3849 + 10kb C-T, R334W, P67L are associated with borderline sweat tests.[6] In these cases the sweat sodium concentration is often higher than chloride and patient is pancreatic sufficient. The diagnosis then remains uncertain in those patients with clinical features of CF. Very rarely, the sweat test can be normal in a patient with a CF genotype.[7]

There have been reports of certain conditions producing false positives in sweat testing and these include, Adrenal insufficiency, anorexia nervosa, atopic dermatitis, autonomic dysfunction, coeliac disease, ectodermal dysplasia, G6PD deficiency, glycogen storage disease type I. hypogammaglobulinemia, hypoparathyroidism, hypothyroidism, Klinefelter's syndrome, malnutrition, mucopolysaccharidosis type I, nephrogenic diabetes insipidus, nephrosis, and pseudohypoaldosteronism.[8,9] In adults and older children the mineralocorticoid suppression adaptation sweat test has been used to try and discriminate between an equivocal and a negative sweat test. Patients undergoing this test receive oral fludrocortisone, (dose; children 3 mg/sqm/ day for 2 days, adults 5mg a day for 2 days) prior to pilocarpine iontophoresis. In healthy individuals the sweat Na values have been reported to fall significantly after fludrocortisone priming.[10] However the recent UK guidelines for the performance of the sweat test suggest that there is no routine place for this test due to paucity of data.

5. Conflict of Interest

None.

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