Original Article

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Comparison of Classic Sweat Test and Crystallization Test in Diagnosis of Cystic Fibrosis

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Abstract

Objective: Sweat chloride measurement is considered a standard diagnostic tool for cystic fibrosis (CF). This study was performed to compare sweat chloride values obtained by quantitative pilocarpine iontophoresis (classic test) with sweat crystallization detected by direct observation of a drop of perspiration under light microscopy in patients with and without CF.

Methods: The tests using both techniques were performed simultaneously in patients with and without CF. Cutoff values of ≥60 mmol/L of chloride concentration for the classic sweat test was considered for diagnosis of CF. In crystallization method, observation of typical dendritic forms of salt crystals under light microscopy was interpreted positive.

Findings: Sixty patients suspected to CF (31 males and 29 females) with age range of 9 months to 2 years underwent the sweat test using both techniques. Median sweat chloride values was 26.13+10.85 in group with negative and 72.76+12.78 mmol/L in group with positive sweat test, respectively. All the patients who had positive sweat test in classic method showed typical dendritic forms of salt crystal in sweat crystallization test, which provided the test with 100% sensitivity (95%CI: 93.1-100). Only one of the 31 subjects with negative results for classic sweat test had positive result for crystallization sweat test, which provided the test with 96.7% specificity (95%CI: 92.9-100). Time spent to perform the crystallization test was significantly shorter than the classic method whereas its cost was also lower than the second method.

Conclusion: There was a good correspondence between two studied methods of sweat test. These results suggested the sweat crystallization test as an alternative test for detecting CF disease with high sensitivity and specificity.

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Key Words: Cystic fibrosis; Diagnosis; Sweat chloride; Sweat crystallization

Introduction

Cystic fibrosis (CF) is the most common autosomal recessive disorder among Caucasians with an incidence rate of 1 in 2,500 individuals[1]. The epithelial cells of several organs, including

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respiratory tract, exocrine pancreas, intestine, vas deferens, hepatobiliary system and also exocrine sweat glands are involved in CF. Therefore several clinical features, including suppurative lung disease, pancreatic insufficiency, neonatal bowel obstruction (meconium ileus), multifocal biliary cirrhosis, absent vas deferens to malabsorbtive condition and growth retardation could be seen in affected patients [2,3].

High sweat electrolytes (chloride and sodium) concentration, which is seen in CF patients became basis for sweat chloride test since 1949 [4]. The measurement of sweat electrolyte concentrations was established as a standard procedure for diagnosis of CF and remained the gold standard test for the diagnosis of CF [5].

The diagnosis of CF could easily be made in the majority of cases based on typical clinical features and abnormal sweat chloride values. In such situations, genetic analysis of Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene is not necessary. However, it may be useful in confirming the diagnosis, which also enables carrier testing and prenatal diagnosis [6,7].

The universally accepted reference intervals for sweat chloride concentrations regardless of age or sex are >60 mmol/L, which is considered diagnostic of CF; 40-60 mmol/L is considered borderline, whilst <40 mmol/L is normal. In addition to sweat chloride concentrations, sweat sodium is also usually measured, as there is little difference between sweat sodium and chloride concentrations [8-10]. In order to ensure accurate results from a quantitative sweat test using filter paper, a minimum sweat rate of 1g/m^2/min corresponding to 75 mg collected in 30 minutes is required (for a 2×2 inch filter paper)[9]. Conventionally, it takes around 45 minutes to collect proper volume (50-400 mg) sweat for accurate test, which makes the classic sweat test as a time consuming procedure. Indeed many local laboratories in developing countries have not been approved to do the test for both techniques of sweat gathering and also electrolytes measuring. Forming of salt crystallization of perspiration described by Ferre Calvete et al [11] could be considered as a useful and alternative test for easy detecting CF patients in these regions. Therefore, we designed this study to compare the results obtained by these two methods.

Subjects and Methods

In this study, 60 children with clinical signs suggestive of CF, who were referred to the Children's Medical Center, Pediatrics Center of Excellence in Iran, were investigated.

The study protocol was approved by the Research Ethics Board of the Children´s Medical Center, Tehran University of Medical Sciences. All children's parents gave their written informed consent.

Classic sweat tests (Gibson and Cooke sweat test) and crystallization test were performed for each subject for at least two times in the referral laboratory of the Hospital.

Localized sweating was produced by iontophoresis of pilocarpine nitrate (Gibson and Cook method) using Wescor gel discs [5,12]. A copper electrode was then attached and a weak electrical current of about 3 milli-amperes (mA) was generated using a 9-volt battery for 5 minutes to stimulate sweating. Immediately following stimulation, a preweighed filter paper was placed directly over the site of the positive electrode. At the end of the collection about one hour later, the filter paper was removed and the weight was determined. The collected sample was then analyzed for quantitative chloride and sodium concentration. The test was repeated for two or three times in all subjects to confirm the results. Meanwhile one drop of each sample was put on lamella and heated by the light of microscope for 5 minutes. Lamella was evaluated for crystal formation using light microscope by an expert pathologist. A photograph was taken for each sample to document further assessment. *P*-value of less than 0.05 was considered significant.

Findings

Sixty children (29 females and 31 males) with age range of 9 months to 2 years had taken part in this

study. The mean sweat chloride values in the female group was 50.24 mEq/L, while it was 49.81mEq/L in the male group without any significant difference between genders (*P*>0.05).

Thirty one subjects including 17 males had negative sweat test. CF was diagnosed for the remaining 29 patients who showed positive values of sweat classic test. Median sweat chloride values of 26.13+10.85 and 72.76+12.78 mmol/L were detected in non-CF and CF patients, respectively.

All of the CF patients had positive sweat crystallization (fig. 1), which provided the test with 100% sensitivity ($^{95\%}$CI: 93.1-100). Only one of 31 subjects without CF (17 males and 14 females; aged 9 months to 2 years) had positive crystallization test, which provided the test with 96.7% specificity (95%CI: 92.9-100).

Discussion

Cystic fibrosis (CF) is one of the most frequent (1 in 2500) autosomal recessive diseases characterized by substantial clinical heterogeneity[13]. Recent studies have begun to identify chromosomal locations that identify specific genes contributing to this variation. Over the past several decades, there has been substantial progress toward diagnostic tools of CF.

Determination of chloride concentration in sweat is the current diagnostic gold standard for CF[14].

The conventional sweat test with elevated sweat sodium and chloride concentration after iontophoresis of pilocarpine is the standard laboratory test for CF. An accurate sweat test relies on coordination of several factors. Technical error of instrument calibration and result reporting are major factors that affect the results. The tests should be performed by expert personnel to ensure sufficient sweat volumes and proper use of equipment. The centers doing such tests should follow standard guidelines to reduce complexity in interpreting a variety of result ranges [14,15]. Advanced equipments and experienced personnel which are necessary for accurate classic sweat test made this test unavailable for many centers, especially in developing countries. Moreover, collection of sweat for some infants is difficult and time consuming.

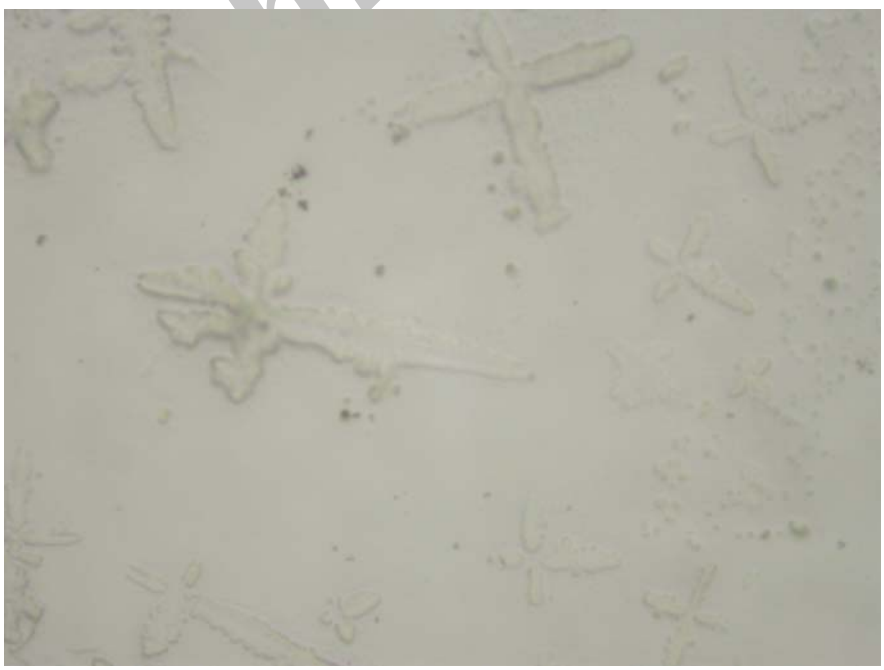

Fig. 1: Crystal formation in sweat sample of Cystic Fibrosis patients

Although sweat studies became standard diagnostic strategy for diagnosis of CF, it has some limitations whilst it may be unreliable due to not enough collected sweat or borderline values. Indeed genetic studies to detect CFTR mutation(s) take time and may even find no useful information. Therefore selecting the best cost-benefit method with high sensitivity and specificity is needed for diagnosis of CF.

For this reasons Nanoduct as a new analyzing system measuring conductivity which requires only 3 microliters of sweat and gives results within 30 minutes is also introduced as a reliable diagnostic tool. Nanoduct has a failure rate comparable to other sweat tests and can be used as a simple bedside test for fast and reliable exclusion, diagnosis or suspicion of CF[16]. Sands and his colleagues indicated that simultaneous bilateral sweat testing with two different methods (concentration and conductivity or Nonoduct) provides an extra quality control system, allowing more time efficient organization of the diagnostic and training procedures[17].

Forming of salt crystallization of perspiration[11] seems as an attractive and alternative test for easy detecting CF patients.

In this study, we have shown that looking for salt crystals in just one drop of sweat could diagnose CF, since crystal formation of sweat under light microscope was detected in a significant number of CF patients. Comparing these two methods of sweat test showed good positive predictive value of 96.7% and the negative predictive value of 100% with specificity and sensitivity of 96.7% and 100%, respectively. Therefore, the test could be a very useful alternative test, whenever the classic test is not accessible.

Since the classic sweat test measuring chloride levels with the use of acceptable methods (Gibson-Cooke or Wescor Macroduct) should be performed in centers that conduct sweat tests frequently with properly documented controls, we recommend sweat crystallization test as an alternative test for CF diagnosis at least in areas where neither classic sweat test nor genotyping are accessible. More studies in this field are also needed.

Limitations: There were some limiting factors to consider in interpreting the study's result. First, this study was conducted on a relatively small sample size. Ideally, a larger and more popular sample size would perhaps delineate more suitable differences between the two methods of CF diagnosis in children with cystic fibrosis. Second, this study compared two kinds of test in children whose first presentation was compatible with cystic fibrosis, although this would not be statistically a problem. As further study, comparing two methods of the mentioned tests between CF patients and normal children could be more helpful.

Conclusion

This study demonstrates the validity of sweat crystal formation test to support a diagnosis of CF in children whenever conventional sweat test is unavailable.

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Conflict of Interest: None

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