RESEARCH PAPER

Aquagenic Wrinkling of Skin: A Screening Test for Cystic Fibrosis

ANUJ SINGH[1], RAKESH LODHA[1], SHIVARAM SHASTRI[1], G SETHURAMAN[2], KN SREEDEVI[1], MADHULIKA KABRA[1] AND SK KABRA[1]

From Departments of [1]Pediatrics and [2]Dermatology, All India Institute of Medical Sciences, New Delhi, India.

Correspondence: Dr SK Kabra, Professor, Department of Pediatrics, All India Institute of Medical Sciences, New Delhi 110 029, India. skkabra@hotmail.com

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Objectives: To study the utility of aquagenic wrinkling as screening test for children with cystic fibrosis.

Design: Evaluation of diagnostic test.

Setting: Pediatric Chest Clinic, and Pediatric Wards of a tertiary care hospital in New Delhi.

Participants: Three groups (children with cystic fibrosis, carriers of cystic fibrosis, and controls).

Method: Time taken to develop aquagenic wrinkling was measured. The test was performed by asking the enrolled subject to put their one hand in water and was checked for development of wrinkling every minute, and a photograph was also taken every minute.

Results: A total of 64 children with cystic fibrosis, 64 controls and 64 carriers were enrolled in the study. Median (IQR) time to develop aquagenic wrinkling in the three groups was 2 (1.5,3) minutes, 4 (3,5) minutes and 8 (5,11) minutes, respectively. The optimal cut-off was calculated as 3 minutes by Receiver operating characteristic curve with a sensitivity and specificity for identification of children with cystic fibrosis as 81% and 57%, respectively. The area under curve was 76.5%. The 3 minute cut-off for development of aquagenic wrinkling was applied to 54 children referred for sweat test. 20 children had sweat chloride values of ≥60 mEq/l and diagnosed as cystic fibrosis. 15 of these developed aquagenic wrinkling at ≤3 minutes, giving a sensitivity of 75%.

Conclusion: In places with no facility for sweat test, children with phenotype compatible with cystic fibrosis who develop aquagenic wrinkling in 3 minutes may be diagnosed as probable cystic fibrosis and referred for confirmation by sweat test.

Keywords: *Diagnosis, Sensitivity, Specificity, Sweat test*.

Cystic fibrosis (CF) is the one of the common life-limiting genetic disorders in Caucasians with an incidence of approximately 1 in 2500 children born in the United Kingdom [1]. Previously considered to be rare, recent reports suggest that CF occurs in India and diagnosis is missed [2,3]. The precise incidence of CF among Indian subcontinent is not known. The incidence in migrant Indian populations in the USA has been estimated to be 1 in 40000 [4], same in UK has been estimated between 1 in 10000 to 1 in 12000 [5,6]. A study on 955 cord blood samples reported carrier rate of common mutation c.1521_1523delCTT (also known as DF508) as 0.4% [7]. By using same data the incidence of CF in India is estimated as 1 in 40000 live births.

The prevalence of genetic mutations differs among population subgroups. The commonest mutation is DF508, which is reported between 19 to 44% Indian children [8].

In view of multiple mutations associated with CF, use of genetic mutation testing as diagnostic test for CF is not feasible. With a population of over one billion in India, diagnostic facilities for CF are available in less than ten centers in the country. Even if a clinician suspects CF in a child, he/she is not able to confirm the diagnosis as the patient may have to travel hundreds of kilometers to get the sweat test done. Therefore, diagnosis of CF is not confirmed or gets delayed. It is important to identify children with CF early and administer appropriate treatment.

Accompanying Editorial: Pages 105-6

It has been documented in some studies that children with CF may develop aquagenic wrinkling more rapidly as compared to controls, and has been reported to be a sensitive test for screening children with CF [9-12]; however, it has not been studied in detail. Therefore, we planned the present study to calculate sensitivity and specificity of this simple test as screening test for children with CF.

In this study our primary objective was to document the prevalence of aquagenic wrinkling of skin in patients with cystic fibrosis. Secondary objective was to identify optimal time for aquagenic wrinkling for cases and

carriers, study utility of aquagenic wrinkling of skin as screening test for cystic fibrosis and to determine genotype-phenotype correlation for aquagenic wrinkling in children with cystic fibrosis.

METHODS

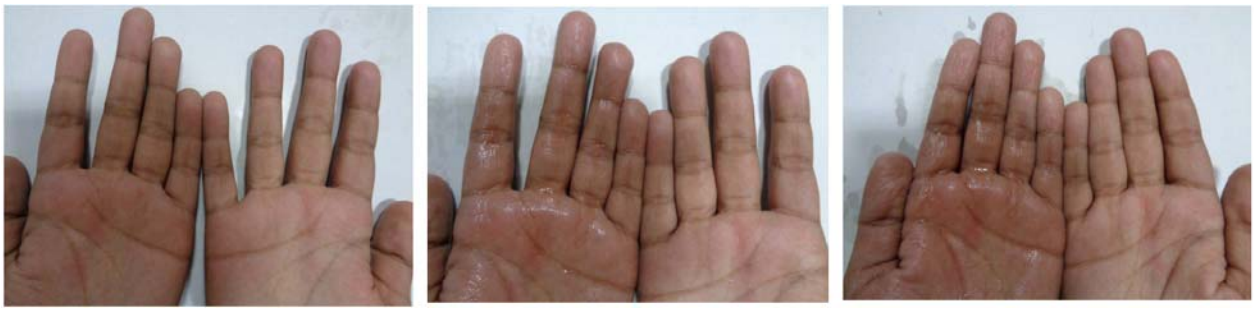
Study design was descriptive study for evaluation of diagnostic test, and conducted at Pediatric Chest Clinic and Pediatric Wards at a tertiary-care center in Northern India. The study was conducted from July 2014 to March 2016 for a total duration of 21 months. There were three groups of participants as cases, control and carriers. Cases were children with cystic fibrosis age >12 months diagnosed with clinical phenotype and sweat chloride >60 mEq/L on two occasions. Carriers were biological mother or father of children with CF. Controls were children without respiratory symptoms attending the pediatrics outpatient department. Exclusion criteria were participants who received aminoglycoside antibiotics in past one month, or those having other dermatological condition affecting palms.

For calculating sample size, it had been documented that aquagenic wrinkling is observed in >53-84% of children with CF in <5 minutes. We presumed the prevalence as 65% and precision of 12%, sample size calculated to be as 64 children with cystic fibrosis. Children referred for sweat chloride during study period were also enrolled for validating the study results.

Eligible participants were enrolled in the study after informed consent. Details for each patient including demographic profile, mutation analysis for cystic fibrosis, and sweat chloride result were recorded. Examination of both hands was done and images were taken (16-megapixel camera) to compare the result prior to immersion in water. RO (Reverse osmosis filter) purified water was used at temperature (25-30^{o}C) in clean standard size tray and kept on level surface. The study participant was asked to immerse one hand in water and time noted as zero. Hands were examined for 3 seconds after every minute to look for wrinkling till 5 minutes and photographed till wrinkling appears on finger tips or palm (***Fig*. 1**). If wrinkling did not appear till 5 minutes, hands were examined every minute and photographs were taken at appearance of wrinkling. Those who did not develop wrinkling by 15 minute were assigned a wrinkling time of 15 minute and photograph taken at 15 minute time. At appearance of wrinkling (by comparing with other hand outside water), time was recorded and photograph was taken. Carriers and controls were examined in a similar fashion.

Outcome variables were defined as aquagenic wrinkling *i.e.* wrinkling of skin on immersion of hand in water that is taken as end point as assessed by observer. Time to aquagenic wrinkling was defined as time from immersion of hand in water to the development of wrinkling of skin of hand by comparing with other hand. Study protocol was approved by institutional Ethics Committee. Participants were enrolled after obtaining a written informed consent from participants/parents or guardians of children.

Statistical analysis: Data were managed using Microsoft Excel and analyzed using Stata 11.0 software. The prevalence of aquagenic wrinkling in CF was the proportion of children positive for aquagenic wrinkling in 5 minutes. The prevalence was compared with that in carriers and controls. Sensitivity and specificity of aquagenic wrinkling at different time intervals was calculated using sweat test as diagnostic gold standard for CF. Mean and median time for development of wrinkling was calculated. Mean time for wrinkling in CF children with positive DF508 and negative for DF508 were compared. For determination of optimal cut off time, Receiver operator characteristic curve (ROC) was drawn.



(*a*) (*b*) (*c*)

FIG. 1 *showing hands at (a) start and (b) at 1 minute of immersion of left hand in RO water in standard tray. Figure 1(c) shows (aquagenic wrinkling of left palm and taken as end point by observer) at end of 2 minutes of immersion in the same child.*

RESULTS

A total of 64 children with cystic fibrosis, 64 controls without clinical symptoms of cystic fibrosis, 64 parents of children with CF were enrolled over a period of 21 months. The median (IQR) age for cystic fibrosis cases was 9 (5-13.7) years; 43 (67.2%) were boys and 21 (32.8%) were girls. Median (IQR) age of controls is 6.5 (5- 8) years; 44 (68.7%) boys. The median (IQR) age of 64 carriers (biological parents of children with cystic fibrosis) was 36 (33-40) years; 30 (46.8%) were males. Fifty-four children, referred for sweat chloride test, were also enrolled; their median (IQR) age was 6 (4-10) years (70.4% boys).

All the 64 enrolled children with CF developed aquagenic wrinkling within 8 minutes. Sixty-one children developed aquagenic wrinkling by 5 minutes, giving a prevalence of aquagenic wrinkling by 5 minutes as 95.3% (95% confidence interval of 90.1- 100%). The prevalence in carriers and controls using the 5-min cut-off was 29.7% and 82.8%, respectively (*P* value for comparison of prevalence in the 3 groups <0.0001). Median time (IQR) for wrinkling in children with CF was 2 (1.5- 3) min while same in controls was 4 min (3-5) and in carriers was 8 min (5-11), respectively. The difference was statistically significant suggesting that it can discriminate between different groups (*P*=0.0001) (***Fig.* 2**).

Sensitivity and specificity of aquagenic wrinkling were calculated by using different cut-off time in cases and controls. Sensitivity for identification of children with CF if they developed wrinkling at one minute was 25% and increased to 100% at 8 minutes. Similarly, specificity at cut-off of one minute was 95% but decreased progressively and was 5% at 8 minutes. Receiver operator (ROC) curve for different cut-offs of time to aquagenic wrinkling was drawn and it suggested an optimal cut-off at 3 minutes with a sensitivity of 81.2% and specificity of 56.2% (***Fig.* 3**). The area under curve was 0.76.

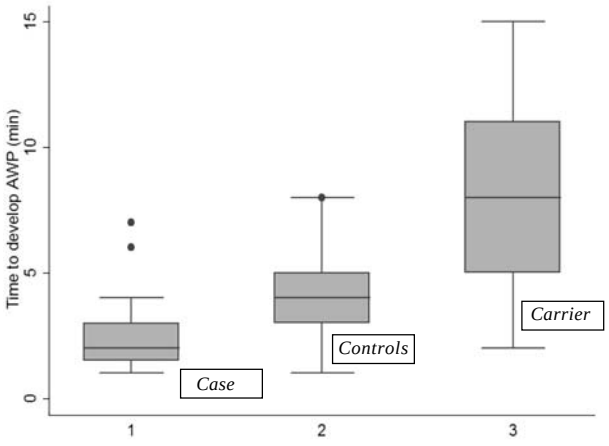


FIG. 2 *Time to develop aquagenic wrinkling in children with cystic fibrosis, controls and carriers of cystic fibrosis gene mutation.*

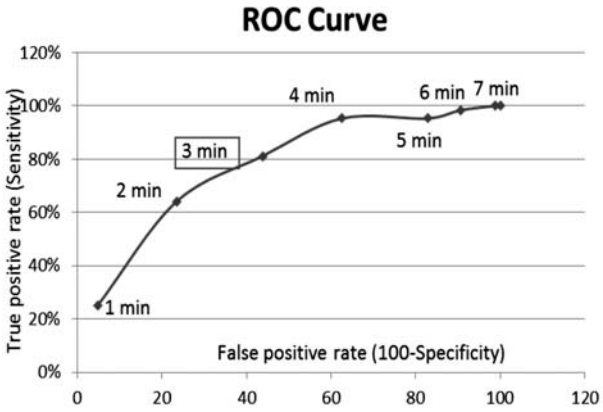


FIG. 3 *Receiver Operating Characteristic curve for time to develop aquagenic wrinkling of palms as a screening test for cystic fibrosis.*

Time to develop aquagenic wrinkling was checked in 54 children referred for sweat chloride test. A total of 20 children had a sweat chloride value of ≥60 mEq/L. Of these 20 children, 15 developed aquagenic wrinkling by 3 minutes. Considering sweat chloride value of ≥60 mEq/L for diagnosis of CF, sensitivity of aquagenic wrinkling of palms by 3 minute was 75%. A total of 13 (20%) children were positive for DF508 and 51 (80%) were negative for mutation. Median (IQR) time of wrinkling in DF508 mutation positive cases was 2 (1- 2) min and in mutation negative cases was 2 (2- 3) min (*P*=0.04). In each age subgroup (less than and more than 6 year age), cases developed aquagenic wrinkling of palm at statistically significant less time as compared to control group. For cases there is trend towards aquagenic wrinkling at less time in subgroup ≤6 year of age (***Table* I**).

TABLE I AQUAGENIC WRINKLING OF PALMS IN CHILDREN WITH CYSTIC FIBROSIS AND CONTROLS

Age category	*Median (IQR) time to develop aquagenic wrinkling of palms; min*			
	n	*Cases*	*n*	*Controls*
Age ≤6 y	23	2 (1- 3)	32	4 (2.5- 5)
Age > 6 y	41	2 (2- 3)	32	4 (3- 5)

P<0.01 for comparison between cases and controls for both age-groups; For comparison between the two age-groups, P=0.43 for cases and P=0.45 for controls.

WHAT IS ALREADY KNOWN?

- Children with cystic fibrosis may develop aquagenic wrinkling faster than normal children.

WHAT THIS STUDY ADDS?

- Aquagenic wrinkling can be used as a potential screening test for cystic fibrosis in children.

DISCUSSION

In this diagnostic test evaluation study, we found that the prevalence of aquagenic wrinkling in children with CF was 95%. We observed that the median time to develop aquagenic wrinkling in children with cystic fibrosis is significantly less as compared to controls (children without CF) and in carriers (parents of CF). Aquagenic wrinkling by 3 minutes had a reasonable sensitivity and specificity for identification of children with cystic fibrosis of 81% and 56%, respectively. Children with CF having DF508 mutation had lower time to aquagenic wrinkling as compared with those negative for DF508 mutation.

Our study had some limitations. The cut-off to define wrinkling of skin was based on observer decision. However, the observer had been trained and supervised; photographs were also taken. In carrier population mutation analysis is not available for all and we assumed parents of CF children as heterozygous for mutation. There is also possibility that the control group may have carriers as we have not done mutation analysis in control group to rule out the same.

Aquagenic wrinkling in CF was first reported in 1974 [13]. After this observation, there were only few case reports until 2004 when Katz, *et al*. [14] reported DF508 homozygous cases presenting with aquagenic wrinkling. Berk, *et al*. [9] did a blinded comparison of 44 CF cases and 26 controls for aquagenic wrinkling of palm by using tap water and 3 minute as cut-off time and gave aquagenic wrinkling score to each participant from score 0 to score 4, and found that mean score was significantly higher in CF cases than in control group. In our study the maximum sensitivity was at 3 minute, although we did not use any aquagenic wrinkling score which might be a more reproducible parameter, but we identified wrinkling at predefined time points after immersion. Gild, *et al.* [10] did a case control study and enrolled 21 patients, 13 carriers and 15 controls and patients with a time to wrinkling of ≤3 min were defined as having aquagenic wrinkling. Mean time to wrinkling was 2 min in cases, 7 min in carriers and 11 min in controls which was comparable to our study. All carriers in our study were not having genetic diagnosis and we enrolled parents of CF cases as carriers, their median time for wrinkling of skin is also comparable. CF carrier having higher time to aquagenic wrinkling can be explained by heterozygous state and normal chloride conductance, in contrast to CF patients. Also median age of carrier population is significantly higher than cases and control which can affect detection of wrinkling in this group. Arkin, *et al*. [11] in a prospective observation trial found higher prevalence of aquagenic wrinkling of the palms in CF cases as compared to controls with no genotype phenotype correlation. In our study we found that children with CF having DF508 mutation had lower time to aquagenic wrinkling as compared with those negative for DF508 mutation. The variation in aquagenic wrinkling time with mutation analysis may be due to possible role of chloride conductance channel role in wrinkling of skin, but as exact pathophysiologic mechanism of aquagenic skin wrinkling is not known so there may be role of other cell volume regulation mechanisms [15]. Chinazzo, *et al*. [12] did cross sectional study in 2014 on 58 children with CF and 23 carriers and seven controls and found that aquagenic wrinkling of palm is more common in CF cases as compared to carrier. No correlation was found between CF genotype and AWP score severity. Garcon, *et al*. [16] did a cross sectional study to determine the frequency of aquagenic palmoplantar keratoderma in 27 CF cases for 2-3 minute in water and found that 41% developed wrinkling of skin. In our study, we had a higher percentage of 81% CF cases developing wrinkling within 3 minutes of hand immersion. Results are different possibly due to shorter time of immersion as compared to our study.

None of the previous studies have evaluated the utility of aquagenic wrinkling of skin as a screening test. In current study, we could document a sensitivity of 75% in children referred for sweat test. Data also suggested that there is a trend towards earlier wrinkling of skin in younger children with cystic fibrosis. This can be possibly attributed to characteristics of skin in younger children which helps in early identification of wrinkling by observer.

Based on observations in our study aquagenic wrinkling of skin by 3 minutes can be used a screening

test and can help for early identification of patients and their referral for diagnostic investigations. In India, where diagnostic facilities for cystic fibrosis is available at few centers, aquagenic wrinkling of skin can be used as a clinically useful screening test. Strengths of our study include enrolment of cases, controls and carriers. We tried to perform test using water with similar electrolyte content (RO water), temperature and compared development of wrinkling with other hand that was not immersed in water. We also took photographs every minute to ensure quality control.

We conclude that children with cystic fibrosis develop aquagenic wrinkling faster than the healthy subjects. Aquagenic wrinkling has a potential to be a simple, inexpensive method to use as screening test for identification of children with CF and especially, in resource limited situation, it may help in early referral and diagnosis, which could translate to decreased morbidity of CF patients.

Contributors: AS: developed protocol, collected data, analysis of data and writing of manuscript; RL: involved in development of protocol, analysis of data and manuscript writing; SS: involved in study development, genetic studies and manuscript writing; GS: involved in development of protocol, monitoring and manuscript writing; KNS: involved in data collection; MK: involved in protocol development, genetic studies and manuscript writing; SKK: involved in protocol development, analysis and manuscript writing. He will act as guarantor for the study.
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