Cystic Fibrosis Diagnosed Using Indigenously Wrapped Sweating Technique: First Large-Scale Study Reporting Socio-Demographic, Clinical, and Laboratory Features among the Children in Bangladesh A Lower Middle Income Country

ARM Luthful Kabir, MBBS, FCPS[1], Sudipta Roy, MBBS, FCPS[1],
Rahat Bin Habib, MBBS, MD[2], Kazi Selim Anwar, MD, MPhil[3],
Md. Abid Hossain Mollah, MBBS, FCPS, Dip. in Med Edu, FRCP[4],
Ruhul Amin, MBBS, FCPS[5], Al Amin Mridha, MBBS, MD, FCPS, FRCP[6],
Jasim Uddin Majumder, MBBS, MD, FCPS[7], Md. Delwar Hossain, MBBS, FCPS[8],
Nazmul Haque, MPhil[4], Shakil Ahmed, MBBS, MD, FCPS[6],
and Mohammod Jobayer Chisti, MBBS, MMed, PhD[9]

Abstract

Due to lack of robust data on childhood cystic fibrosis (CF) in Bangladesh we sought to evaluate their clinico-epidemiology. A cross-sectional observation was conducted adopting CF-foundation consensus-panel-diagnostic criteria in 3 tertiary-care-hospitals in Bangladesh from 2000 to 2017. Clinically suspected 95 CF-cases were subjected to sweat-chloride testing using locally-developed a fast, cheap and effective indigenously body-wrapped sweating technique measured by US-Easy Lyte-automated microprocessor-controlled analyzer marking ≥ 60 mmol/L as positive. Mean-age of CF-cases at disease-onset was 16.9 ± 26.6 months that significantly differed with age-at-diagnosis ($P < .02$). Pulmonary syndromes included chronic wet cough in 100%, respiratory distress in 90.5%, digital-clubbing in 78%, mucopurulent-sputum in 74%-cases, and crepitation in 82%. Radio-imaging revealed bronchiectasis in 60%, hyperinflation/peribronchial-thickening in 22% and, pan-sinusitis in 89%-cases. While 37% had history-of malabsorption, high-fecal-fat revealed in 53%-cases. Malnutrition prevailed as severe-underweight in 87%-cases and all CF-cases (100%) had high sweat-chloride (mean $= 118 \pm 53.34$ mmol/L). Thus, children with pulmonary features coupled with severe malnutrition and associated radio-imaging bronchiectasis should be screened for CF with a fast, cheap and effective sweat test in resource poor settings.

Keywords

Cystic fibrosis, children, first large-scale report, indigenously wrapped sweating technique, Bangladesh

Introduction

Cystic fibrosis (CF) an autosomal recessive (genetic) disorder caused by mutations in both copies of gene encoding for CF-transmembrane conductance regulator (CFTR) protein.[1] This life-limiting disease remain common among the Caucasian population living in America, Europe, Australia and Asia[2–4] though found in non-Caucasian,[5] ethnic minorities,[6] too. The incidence of CF, a life limiting genetic disorder[2] is −1 in 2500 children born in UK, but less common in African-Americans (1/15 000) and Asian-Americans (1:31 000).[3]

CF has a poor prognosis without treatment, in high income countries multidisciplinary care has led to significant improvements in life expectancy. However, in low income countries it is often endured a bad prognosis with ultimate fatality due to lack of multidisciplinary care.[7] CF-associated symptoms reportedly appear throughout a patient's life span, mostly children[2,8,9] manifesting major overlapping in symptoms over time, varying in degrees of suffering and outcome.[8] In first 2 years of life of children with CF often develop multiple chronic symptoms retaining to recurrent pneumonia, productive cough, resistant asthma, failure to thrive, chronic malabsorption/steatorrhea, etc.[10] But mortality occurs due to chronic pulmonary infections and its complications.[7]

According to Indian experience, because of widespread belief that CF does not occur in Indo-Bangladesh subcontinent, the disease is rarely suspected, and, even if it does, the diagnosis is not always confirmed due to poorly available facilities for diagnosis like ours, as Sharma et al attested.[4] Contrarily, current report shows an increase in CF-cases in Asia, Africa and Latin America.[11,12] So, it is logically presumed that CF cases are either missed by the physicians due to knowledge gap, as our recent observation of a KAP-study[13] or often delayed by parental lack of awareness in seeking doctor's advice in time- that Kabra et al assumes due to lower suspicion index.[4,5,14-16]

Unfortunately, to our knowledge there is no organized data-base system for CF in developing countries including Bangladesh until now, mainly due to non-existent of broad-based research-which Ahasan et al pointed out rightly[17] and because of limited knowledge and skill on CF (among physicians). Thus, it is perceived that a lot of children with CF remain undiagnosed and do not receive structured management of CF and succumb to death. Diagnosis of CF is important to alleviate parental agony through plummeting child's sufferings. Though parents get shocked and upset initially, they gradually adjust with the fact thus ceasing to change consultants frequently which cut down huge expenses of their hard-earned mid-class income. Instead, they may attend a specified center to pursue the optimized treatment plans, once available in a country. That is what we are currently thriving for. Since precise estimation of CF neither exists nor being tried yet in Bangladesh we, conducted this study with the aim to bring out principal clinical features of childhood-CF. We also aimed to figure out approximate (if not exact) time lag between disease-onset and diagnosis. The results of our analyses may help clinicians to use our fast, cheap and effective approach for the potential diagnosis and management of children with CF especially in resource poor settings like Bangladesh.

Methods

Ethical Approval and Informed Consent

The study was approved by the Institutional Review Board (IRB) of the Institute of Child and Mother Health (ICMH), Matuail, Dhaka, Bangladesh. The reference of the approved protocol is ICMH/IRB-11FEB2020/025. The informed consent was obtained from the caregivers or parents of the patients prior to the enrolment into the study.

Set Up and Patients

This long-term study was based on a series of cross-sectional observations over the last 17 years on chronic pulmonary disease plus positive sweat chloride test on one occasion.[18] We utilized a unique patient selection method that consisted of 3-tiers of screening process: 1st tier: suspected CF-cases identified; 2nd-tier: stringent differential diagnoses, anthropometry and radio-imaging done; and 3rd tier: presumptively identified CF-cases were confirmed using a pre-tested non-invasive sweat-chloride test-procedure.

[1]Ad-din Women's Medical College Hospital, Dhaka, Bangladesh
[2]Director General Health Services, Dhaka, Bangladesh
[3]IUHW, Narita, Chiba, Japan
[4]Dhaka Medical College, Dhaka, Bangladesh
[5]Institute of Child Health (BICH), Dhaka, Bangladesh
[6]Shaheed Shuhrawardy Medical College, Dhaka, Bangladesh
[7]East West Medical College, Dhaka, Bangladesh
[8]Institute of Child and Mother Health (ICMH), Dhaka, Bangladesh
[9]International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh

Corresponding Author:
Mohammod Jobayer Chisti, Senior Scientist & Head, Hospitals, & Clinical Lead, ICU, Dhaka Hospital, icddr,b, Dhaka Hospital, 68 Shahed Tajuddin Ahmed Sarani, Mohakhali, Dhaka, 1212, Bangladesh.
Email: chisti@icddrb.org

Project /Research Design

Study Type: Hospital-based study on series of cross-sectional observation on pertinent clinico-epidemiological features of CF among Bangladeshi children.

Study place: Pediatric wards of purposively selected 3 national level tertiary care hospitals in Dhaka, Bangladesh: (i) Institute of Child and Mother Health (ICMH), (ii) Sir Salimullah Medical College and Mitford Hospital (SSMC-MH) and, (iii) Ad-din Women's Medical College and Hospital (AWMCH).

Study Period: 17 years (Dec 2000-2017).

Study Population: Any child irrespective of sex & socio-economic status hospitalized with suspected CF.

Inclusion Criteria

Following the Cystic Fibrosis Foundation (CFF) consensus panel diagnostic criteria any hospitalized children having chronic pulmonary disease (persistent cough, productive/purulent sputum and persistent/recurrent pneumonia with pulmonary infiltrate or bronchiectasis on CXR/CT-scan) were included.

Exclusion Criteria

Children with suggestive clinical features of bronchial asthma, aspiration syndrome/GERD, pulmonary tuberculosis, foreign body aspiration, primary immunodeficiency, congenital heart disease, pulmonary abscess, congenital diaphragmatic hernia and congenital lobar emphysema were excluded from this study.

Training of Research Officers

Two junior pediatricians employed at each of 3 hospitals to record details of all CF-cases, informing those to the concerned doctors (Co/PIs) to establish final (accurate) diagnosis.

Selection of Study Children as CF-cases

Following CFF consensus panel diagnostic criteria, all the 224 CF-suspected cases were initially enrolled in 3 phases from where 198 cases (88%) were primarily selected for sweat chloride test and 26 (12%) were not. Of these 198, sweat chloride test could not be done on 4 children because of being very sick or getting no consent and from the remaining 194 children 99 (51%) were found to be negative for CF ($\leq$40 mmol/L as 60 mmol/L was used as the sweat testing cut off)[19] and 9/99 had intermediate value (40-59 mmol/L).[20] The rest 95 cases (49%) finally constituted as confirmed CF cases having their sweat-chloride level $\geq$60 mmol/L measured on automated electrolyte analyzer (Figure 1).

Research Instruments Used

Diagnostic Method

A pretested structured questionnaire was used to fill up for all patients following an interview with the parents. Chest X-ray (CXR) and stool for fat droplets and other investigations as indicated were done in all cases. Besides, the supportive laboratory tests like blood counts, throat swab, blood culture, high resolution computed tomography (HRCT) scan of chest, X-ray para nasal sinus (PNS), barium esophagogram, gastric lavage for acid fast bacilli (AFB), Mantoux test (MT), electro-cardiography (ECG), echocardiography, primary immunodeficiency (PID) panel, saccharine test and fiber optic bronchoscopy (FOB) were performed whenever indicated.

Procedure of Data Collection

Demographic and socio-economic data were collected administering a face-to-face interview with the parents of affected children using a hybrid-designed questionnaire (open and close-ended questions) including detailed history, thorough physical examination and in-depth anthropometric analysis. The children were subjected to routine investigation, HRCT of lungs and other investigations as mentioned above whenever indicated. All the suspected CF cases were subjected to sweat chloride test.

Clinical Case Management Protocol

This was prepared in line of therapeutic management. Treatment included counseling about the disease, airway clearance technique, antibiotics, anti-inflammatory agents, mucolytics, pancreatic enzyme replacement, calcium, multivitamins and nutritional support.

The Co/PIs in adjunct to pre-trained research officers also followed up the post-treatment status at the pediatric OPDs on given dates (once in 2-3 months at the least) in respective hospitals or through cellphone-based enquiry, once the patient's guardians remain outside Dhaka.

Sweat Test: Method of Measuring Sweat Chloride to Confirm Cystic Fibrosis

Indigenously Wrapped Sweating Technique

Taking the idea of gold standard test for diagnosing CF[21] we developed our own method of "Indigenously Wrapped Sweating Technique" for the collection of body sweats due to unavailability of pilocarpine-iontophoresis test. This is completely a non-invasive method, being fast, cheap, and yet effective and acceptable both by the child and parents. This procedure was pre-tested

prior to applying this on our CF-cases. For sweat test the following materials were used: a piece of polythene, IV fluid, pulse oximeter, oxygen cylinder with flow meter, room heater, test tube, and auto-analyzer.

This indigenously wrapped sweating technique was conducted in the hospital ward in following 3 steps:

1st Step: Preparing the Patients Who Were CF Suspected

Counseling both the guardians and child. The parents of CF-suspected children were first explained on the methods in good details and then counseled both the guardian and older child to create in-depth rapport and effective compliance. Only then all the vital signs and overall health status was first assessed thoroughly.

2nd Step: Collection of Body Sweat Using Indigenously Wrapped Sweating Technique

Patient's whole body was first washed with boiled and cooled water and properly dried. Then the body of child was wrapped with a long piece of polythene. Then a blanket (in winter only) was put on top of it to keep the child's body warm enough. However, child's face was kept open to allow breathing and essential support of finger pulse-oximeter to facilitate monitoring oxygen saturation.[22] This indigenous wrapping technique was able to produce at least 1 to 2 ml sweat (minimally required for sweat analysis as mandatorily). However, this remains the first of its type of sweat collection reported in the world.

3rd Step: Analysis of Collected Body Sweat

Collected body sweat (1-2 ml) were finally measured by analyzing sweat chloride using a pre-standardized automated microprocessor-controlled analyzer *(EasyLyte. Medica Corp., 5 Oak Park Drive Bedford, MA 01730-1413 USA).*

Any child producing sweat chloride in an amount of ≥60 mmol/L on a single occasion was diagnosed as CF along with suggestive long-term prevailing clinical features. Sweat chloride ranging between 40 and 59 mmol/L with relevant clinical presentations were subjected to repeat the test. A sweat-test yielding a reading of <40 mmol/L was considered as "normal".[20]

Radiological Imaging

Radiological tests like X-ray chest was done in all cases and X-ray paranasal sinus, high resolution CT-scan (HRCT) of chest were performed whenever indicated.

Other Pertinent Pathological/Lab Tests

Stool Microscopy to find fecal fat: Stool of the patients were sent for fecal fat analysis. The analysis was done in the following way: 5 ml stool was mixed with 10 ml water in a test tube. Small amount of the mixed stool was placed in a glass slide. Two drops of 95% ethanol was mixed. Then 2 drops of saturated solution of Sudan III was added for further mixing. Cover slide was placed and examined under microscope. Up to 60 droplets/HPF is considered normal and more is steatorrhea.

Bacteriological culture of throat swabs were performed in selective CF-cases that comprised nearly half of all (48, 50.5%) CF children: only to determine growth of *Pseudomonas aeruginosa* and/or *Staphylococcus aureus-* which are recognized as a good indicator in favor of CF positivity.[23]

Follow up of study children: All children were asked to come for follow up at a regular interval to assess their existing condition and/or prognosis for further clinical advices if/as required.

Data Management (data collection, entry, analysis, inferences)

All data collected from respective hospitals were first re-checked visually before entering those into an IBM compatible PC by a pre-trained expert data entry technician & was cross-checked by the Co/PIs to ensure the quality of database properly. All the cleaned data were analyzed using SPSS/Win V.22 using appropriate statistical lines. Data were subjected to Pearson's Chi-square/ Fisher's exact test to see proportional association/differences among/between variables having discrete/categorical and/or dichotomous values.

Results

Total 194 (87%) of 224 initially-suspected cases underwent sweat chloride test of whom 49% revealed positive for sweat chloride (≥60 mmol/L) constituting finally as clinically confirmed CF cases.

However, clinical examinations revealed: of 99 children who had sweat chloride test negative, 21 had asthma, 17 had unilateral bronchiectasis and 14 persistent pneumonia. It was followed by 11 each of non-CF bilateral bronchiectasis, GERD, post bronchiolitis infantile wheeze. However, 5 had a history of pulmonary TB and 3 had congenital heart disease with pneumonia. Two each had PCD and suspected childhood interstitial lung disease (chILD). However, 1 each had idiopathic pulmonary hemosiderosis, and congenital pulmonary airway malformation-CPAM (Figure 1).
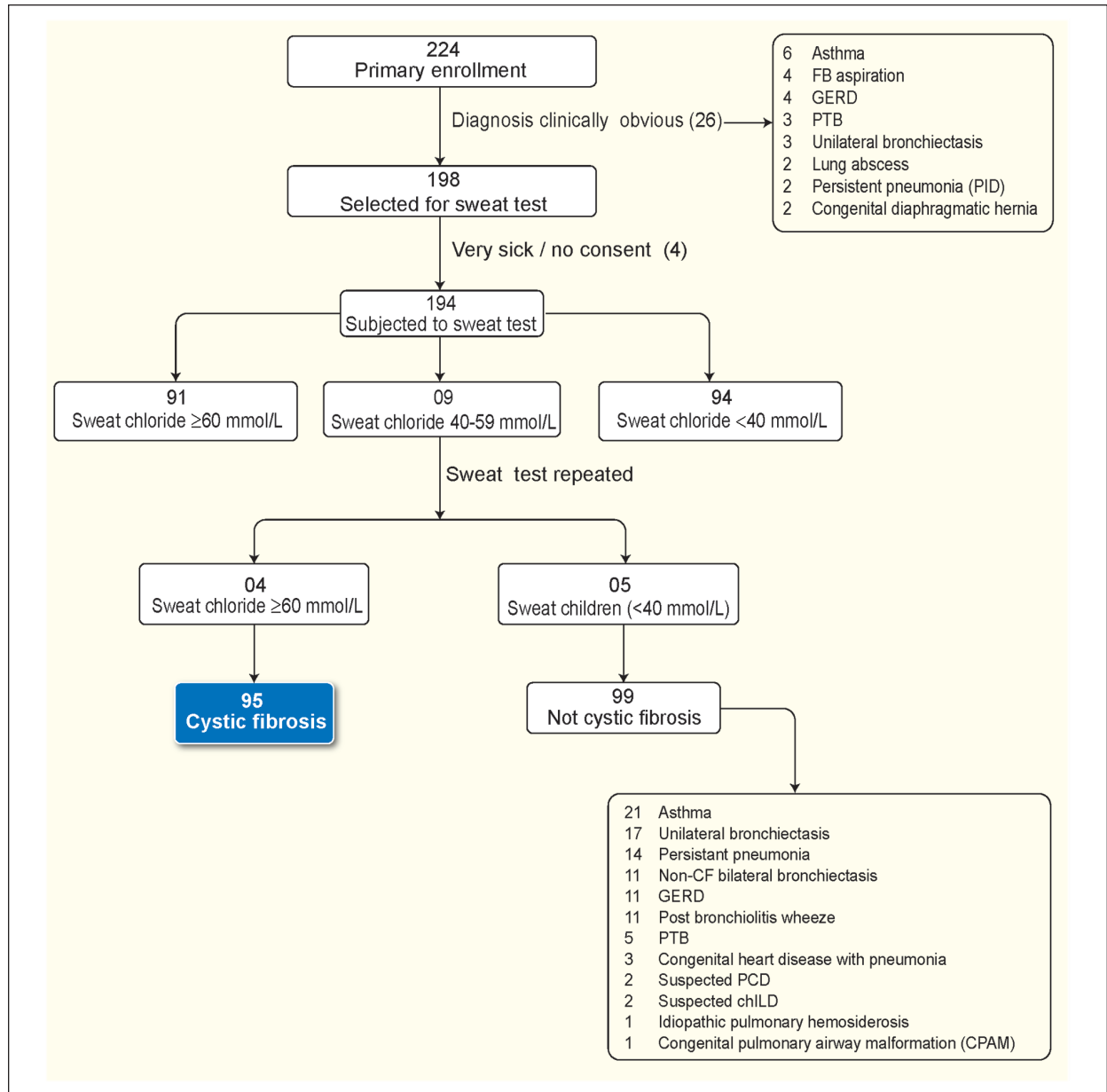
Figure 1. Flow diagram.

Moreover, sweat chloride test was not done in 26 children: 6 of whom had asthma, 4 each had either foreign body aspiration or GERD. Three each had pulmonary TB and unilateral bronchiectasis, while 2 each had lung abscess and persistent pneumonia (due to primary immuno-deficiency), and 2 had congenital diaphragmatic hernia as the diagnoses were obvious.

Findings on Child's Demographic Features and Socio-Economic Status

Age of 95 children with CF at disease onset (mean: 16.9 ± 26.6 months), 74% (70 of 95 younger ones: 56% of 1-6 months-old toddlers and 18% of 7-12 months-old infants) and, 26% (25/95) were >12 months-old. Age at diagnosis (mean: 90.0 ± 48.5 months) revealed –24% (23/95) were under 5 years-old, –53% (50/95) were 5.1 to 10 years age group and the rest 22 (23%) were older boys and girls belonging to 10.1 to 18 years old. Age at disease-onset differed significantly than that of diagnosis. However, child's age at diagnosis either did not differ with sex, religion (88 Muslims vs.7 Hindus: $P > 0.6$, and parental consanguinity (Table 1).

Socio-economically, most children were poor (87.4%) belonging to "Lower to mid" income groups who maintained their livelihood with a very tight monthly average

Table 1. Association of Children's age at Diagnosis with Various Socio-Demographic Characteristics.

Age at disease onset/symptom when the child attend the 1st doctor earlier (Mean age = 16.9 ± 26.6 months)		Child's age (recoded) at diagnosis (n = 95) (Mean age = 90.0 ± 48.5 months)			Statistical significance (P-values: at 95% CI)
		6-60 mons (up to 5 yrs. = 23)	61-120 mons (5.1-10 yrs. = 50)	121-216 mons* (10.1->16 yrs. = 22)	
1-6 months-old infants (n = 53, 56%)		17	29	7	Pearson's X^2:
7-12 months (<1 year-old children, n = 17, 18%)		5	7	5	$P < 0.01$ HS
>12 months (>1 year old boys & girls, n = 25, 26%)		1	14	10	
Sex	Boys (n = 44, 46.3%)	15	21	8	Pearson Chi-Square:
	Girls (n = 51, 53.7%)	8	29	14	$P > 0.10$ NS
Religion	Muslim (n = 88, 92.6%)	19	49	20	Pearson Chi-Square:
	Hindu (n = 7, 7.4%)	4	1	2	$P > 0.06$ NS
Consanguinity	Yes (n = 21, 22.1%)	5	10	6	Pearson Chi-Square:
	No (n = 74, 77.9%)	18	40	16	$P > 0.79$ NS

Statistical significance: HS, Highly; NS, Not significant.
Note: (i) All 95 cases were identified, diagnosed and managed by our research team in our study units at the ICMH, SSMC-MH and AWMCH (2000-2017). None of CF cases were diagnosed elsewhere outside Dhaka either by any pediatricians or other physicians *(Data not shown details, separately).*
(ii) Socio-economic status of most children (87.4%) belonged to lower to mid income groups who were to maintained their livelihood with a very tight monthly budget (on average: 26,679 Bangladeshi Taka (BDT) /currency (equivalent to 315.69 (US\$: As of 8 Sept 2019) *(Data not shown here).*
*Ref: Govt. of Bangladesh. National Children Policy 2011. Ministry of Women & Child Affairs, Feb 2011.

budget of 26,679 BDT Bangladeshi Taka (currency, equivalent to 315.69 US\$). However, detailed socio-economic data of child's family/household income is not shown here, in this paper, except a short foot note under Table 1.

Clinical Findings: History of Disease, its Symptoms and Signs

There was a history of (h/o) persistent/recurrent pneumonia 79 (83%), bronchiolitis 55 (58%) cases, while 16 (17%) received treatment for suspected pulmonary TB (PTB). While 35 had malabsorption (37%), 8 cases had hemoptysis (8.4%). Among all the presenting symptoms, persistent cough predominated among all the 95 CF cases (100%), while respiratory distress was in 90.5% cases (86/95) followed by purulent sputum in 70 (74%) cases. And, 54 cases (57%) each, gave a history of malodor breath and wheezing (Table 2).

Among all signs, the most commonly encountered physical finding was crepitation in 78 (83%), digital clubbing in 67 (78%), chest in-drawing in 54 (59%), followed by rhonchi in 49 (52%) cases (Table 2).

None of the reported symptoms had any significant difference with age at diagnosis, except mucopurulent sputum, and h/o hemoptysis. Importantly, all the 95 children had persistent cough. However, some of presenting physical signs showed significant differences with child's age, like crepitation and chest in-drawing (Table 3).

Table 4, shows the nutritional status of these 95 CF-children in terms of detailed anthropometric analysis based on WHO-recommended Z-score for age specific height and weight measurements including BMI. It revealed 87.3% children were underweight (sever-to-moderate): of whom, 81% were severely underweight (z-score of <-3 SD weight for age: WAZ) and the rest 6% had moderate underweight (<-2 SD WAZ). Overall wasting among under-5 children was >78% (sever-to-moderate). About half (11/23) had severe wasting (Z-score of <-3 SD weight for height: WHZ), the rest 30.4% (7/23) were moderate (z-score <-2 WHZ). Overall stunting (z-score height for age: HAZ) was revealed in 87% (13/23) under 5 children where 56.5% (13/23) were severely stunted, followed by 30.4% (7/23) moderate and the rest 22% (5/23) were mildly stunted. Overall short stature (height below 3rd centile) was assessed as low among 68% (49/72) of all 72 elderly children (>5 years-old). 98.6% children were found to have a BMI of <-3SD in terms of z-score, or, below 5th centile.

Table 5 describes findings on laboratory tests performed to confirm clinically diagnosed CF cases.

Findings of sweat test: Sweat test was found positive in all 95 successively screened out CF cases (100% had a sweat chloride in ≥60 mmol/L sweat), the mean sweat chloride being 118.82 ± 52.3 (range: 60-300 mmol/L). When findings of all positive tests were abridged into 2 dichotomous values: High (60-99 mmol/L) in 42 and very

Table 2. Previous History of Illness, Symptoms and Signs of Childhood-Cystic Fibrosis (n = 95)*.

*[1]Past history of various complaints	Frequency (n = 95)	%
1. Bronchiolitis	55	57.9
2. Treatment received for suspected PTB	16	16.8
3. Recurrent fever	78	82.1
4. Malabsorption	35	36.8
5. Hemoptysis	8	8.4
6. Recurrent/ persistent pneumonia	79	83.2
*[2]Presenting symptoms of CF	Frequency (n = 95)	%
1. Persistent Cough	95	100
2. Purulent sputum	70	73.7
3. Malodor breath	54	56.8
4. Respiratory distress	86	90.5
5. Wheeze	54	56.8
*[3]Presenting signs of CF	Frequency (*n* = 95)	%
1. Clubbing	67	77.9
2. Chest in-drawing	54	58.7
3. Crepitation	78	82.1
4. Rhonchi	49	51.6

*Presenting clinical features among all the 95 CF-cases were examined on diagnosis (lag of 7 years from disease onset).
*[1]Past history of disease (based on parental complains) remain important to hint CF that largely augment in clinical diagnosis.
Presenting *[2]symptoms and *[3]Signs among CF-cases bears crucial diagnostic values.

Table 3. Association of Pertinent Pulmonary Symptoms and/or Signs with Child's Age at Diagnosis.

	Child's Age (recoded) at diagnosis (n = 95) (Mean age = 89.9 ± 48.5 months)			Statistical significance (*P*-values: at 95% CI)	
*[1]Pulmonary symptoms and/or signs	6-60 mons (up to 5 yrs. = 23)	61-120 mons (5.1-10 yrs. = 50)	121-216 mons* (10.1->16 yrs. = 22)	Pearson's Chi-square (χ^2) test	Specific remark/ comment
Persistent cough (95)	23	50	22	*[2]Universal distributed (Constant)	
Mucopurulent sputum (70)	12	38	20	$P < .01$	Highly significant
Chest in drawing (54)	19	27	8	$P < .01$	Highly significant
Crepitation (78)	23	38	17	$P < .04$	Significant
Hemoptysis (8)	1	2	5	$P < .03$	Significant

All (second brackets) indicate total number of CF-cases; *Ref: Govt. of Bangladesh. National Children Policy 2011. Ministry of Women & Child Affairs, Feb 2011.
*[1]All variables are shown in dichotomous (Yes or No) to facilitate running proportional values of significances tests (Pearson's χ^2 with *Likelihood Ratio, and linear-by-linear Association, as well*). *[2]Constant variable: Can't be computed for proportion test for significance for a 2×2 cross-tabulation since the variable "cough" was universally distributed among all 95 cases.

high (≥100 mmol/L) in 53 CF-cases, it yielded a significant difference with age at diagnosis showing proportionately very high levels of sweat chloride among the older group in contrast to younger ones having it at high level (Table 5) potentially due to late diagnosis in older group.

Findings of Radio-imaging

Findings of radiological features on 92 X-ray films/ reports (X-ray report yielded normal findings in 3 cases) revealed all types of hyperinflation, peri-bronchial thickening, bronchial dilatation, cystic lesions, collapse consolidation and nodular densities, etc., where mostly "signet ring" sign along with other features and atelectasis on HRCT were observed more. However, when we broadly abridged all these varieties of X-ray/HRCT findings into 3 major groups, it yielded more meaningful findings. Most of 92 CF-children had bronchiectasis (62%, 57/92) followed by 23% (21/92) all types of hyperinflation +peri-bronchial thickening, and bilateral bronchopneumonia were diagnosed in 15% (14/92) cases. These abridged/grouped radiological findings

Table 4. Assessment of Nutritional Status of CF Children Based on Anthropometric Analysis.

Nutritional parameters used to assess specific nutritional condition	Findings of state of nutrition among children with CF				^Average grades of nutritional status
	Specific nutritional measurement	Z-Scores SD	Frequency	Percentage	
[1]Underweight (*Z-score of weight for age*) (among total 95 cases)	Severe	(<−3 SD)	77	81.0	Very high 87.3% underweight among all CF cases
	Moderate	(<−2 SD)	6	6.3	
	Mild/Normal	(<−1 SD)/normal	12	12.6	
[2]Wasting: (*Z-score of Wt. for Ht.*) (among 23 <5 years-old cases)	Severe	(<−3 SD)	11	47.8	Very high 78.2% of under 5-years had wasting
	Moderate	(<−2 SD)	7	30.4	
	Mild/Normal	(<−1 SD)/normal	5	21.7	
Stunting: (*Z-score of Ht. for Age*) (among 23 < 5 years-old cases) Rest 72 were >5 years ages.	Severe	(<−3 SD)	13	56.5	Very high 86.9% of under-5 years remain stunted
	Moderate	(<−2 SD)	7	30.4	
	Mild/Normal	(<−1 SD)/normal	3	13.0	
Short stature: (*Ht below 3rd centile*) (among 72 >5 years-old cases)	<3rd centile		49	68.0	High 68% >5 years stature
Body mass index: BMI (*Age specific Z-score of BMI*)(Z-score: among 72 children >5 years age)	State of body mass index (BMI):				Very Low BMI 98.6% in terms of z-score and centile calculation
	Measuring Z-score of BMI	Very low BMI	<−3 SD	71	98.6
		Normal BMI	Normal	1	1.38
(Centile: among 72 children >5 years age)	Measuring <5th Centile of BMI	Very low BMI	<5th centile	71	98.6
		Normal BMI	>5th centile	1	1.38

Nutrition profile of children with cystic fibrosis (N = 95).
^USAID, FANTA, FHI360.
[1]Composite measure of both acute & chronic nutritional deficiencies.
[2]Acute nutritional deficiency.

Table 5. Assoc. of Pathological Test and Radiological Images with Child's Age at Diagnosis.

Pertinent clinico-pathological and radiological variables compared	Child's Age (recoded) at diagnosis (n = 95) (Mean age = 90.0 ± 48.5 months)			Stat tests to measure significance (P-values: at 95% CI)	Specific remark/ comment
	6-60 mons (up to 5 yrs. = 23)	61-120 mons (5.1-10 yrs. = 50)	121-216 mons# (10.1->16 yrs. = 22)	Pearson's chi-sq. X^2	
*1Sweat test (level of sweat chloride in mmol/L)					
High (60-99 mmol/L) (42/95)	10	17	15	P < .03	Significant
Very high ≥100 mmol/L (53/95)	13	33	7		
*2Radiological imaging					
Bilateral bronchiectasis (57/92)	0	38	19	P < .00	Highly significant
Bilateral bronchopneumonia (14/92)	4	9	1		
All Hyperinflation + peri-bronchial thickening (21/92)	16	3	2		
Pan-sinusitis (PNS +ve 40/45)*3	3	15	22	P < .00	Highly significant
Stool: microscopy and culture					
Fecal fat (>60) (n = 50)	10	25	15	P > .22	Not significant
Sputum: microscopy and culture					
Bacterial growth*4 *Pseudomonas aeruginosa* (13/48) (n = 48)	2	7	4	P > .85	Not significant

#Ref: Government of Bangladesh. National Children Policy 2011. Ministry of Women and Child Affairs, Feb 2011.
*1All the 95 sweat sample (100%) were positive with a mean of 118.82 ± 52.34 mmol/ml: range between 60 (minimum) to 300 (Maximum).
*2Three chest X-rays had normal findings, but x-ray findings of rest of 92 CF cases differed significantly (50% expected less <5 count).
*3Of 95 CF cases, 45 PNS (X-ray) 47.4% were done, and in rest 50 (52.6%) cases PNS were not done.
*4Two of 13 positive bacterial growth of *P. aeruginosa* in sputum also grew *Staphylococcus aureus* grew as co-infection.

revealed to be significantly associated with child's age at diagnosis. Interestingly, reasons of 3 chest X-ray being normal being presentation in the early phase of life with early stage of the disease (Table 5).

However, 45 cases (–47.4%) who underwent x-ray of paranasal sinuses (PNS) 40 cases (89%) had pan-sinusitis that yielded a highly significantly association with child's age at diagnosis, the elderly groups of CF-cases being more to have had pan-sinusitis (Table 5).

Findings of Other Laboratory Tests

Hematological tests (CBC/blood counts) were done only in few cases only, as deemed necessary, which did not yield any significant findings.

Contrarily, findings of stool microscopy for fecal fat analysis were positive in 53% (n = 50) among all the 95 CF-children and, rest 45 (47%) were negative. However, fecal fat was not associated to differ with age groups significantly.

Findings of Bacteriological Culture and Sensitivity (C/S) of Sputum

Bacteriological C/S of sputum was performed in 48 selective CF cases that comprised a little more than half (50.5%). *P. aeruginosa* were yielded in sputum of 13 (27%) CF-cases. However, C/S was not done in 47 (49%) cases. Interestingly all these positive cultures revealed the growth of *P. aeruginosa*, except in two- which had co-infections with *P. aeruginosa* and *Staphylococcus aureus*.

CF-Case Fatality in Our Study Children

Eleven children of our CF-cases died the cause being respiratory failure except in one who died of profuse hemorrhage (hematemesis and melena).

Discussion

This long-term clinico-epidemiological study on childhood CF study, first time in the country, was conducted

by our pediatric respiratory research group in Bangladesh in 3 tertiary-care hospitals.

Principal Driven Force for Conducting the Study

Unfortunately, neither any organized national database on CF exist in the country nor yet had been any population-based extensive research on CF[14] to allow a precise estimation on it. Several hypothetical considerations based on our earlier observations led us to a hunch that CF was either remained neglected or lacked importance by the general physician/pediatricians[13] *(Personal communication).* We therefore, conducted this 17 years long study to bring out a first-hand comprehensive data on the prevailing clinico-epidemiology of CF that exist in Bangladesh since as recently reported that CF occurs in non-Caucasian[5] and, among ethnic minorities[6] and Indians.[11,12]

Value Added in Conducting this Study

This study also remains unique in its detailed research designing, sound methodological approach and developing a low-cost, rapid but effective indigenously wrapped-sweating technique. Apart from these, we unveiled few cause-effect relationship among child's age at diagnosis and selected clinico-epidemiological features and the consequences, though CF has a known bad prognosis.

While Kabra et al conducted an 8-years long (1995-2002) study in India[11] nothing much was done in Bangladesh, except 3 short drives. While the 1st one remains a simple literature review (2005),[17] the 2nd one was a report on 2 CF-cases only (2016).[24] However, Probir et al gave a 3rd drive in the form of a leading article[25] yet a review. Nevertheless, trouble shooting on relevant issues is yet to be addressed, case-identifications to be practiced and disease diagnosis to be established in Bangladesh like other countries.[11,22]

Thus, data that our study generated will boost physician's knowledge on CF clinico-epidemiology. A recently conducted KAP-study evidenced a gross deficiency in their perceived knowledge on CF among our graduate doctors.[13] Furthermore, findings of this study will contribute to increase mass awareness of our people since most of them remain unaware of CF as Sarker PK et al commented, rightly.[25]

Measuring Sweat-Chloride to Diagnose CF: Rationale of Our Indigenous Method

We conducted this invaluable test for our clinically suspected CF-cases referred to us in respective pediatric respiratory study clinics. Since Gibson-Cooke's standard sweat test[21] require skilled manpower, special care and needs to be performed in accredited laboratories. Earlier in India, Kabra et al[26] in 2002 based on modified Gibson-Cooke's method validated an inexpensive indigenous method for collecting sweat (>100 mg) chloride and titration among 602 Indian CF-patients, sweat of 80% having an average sweat of 230 mg. Kabra et al commented it as acceptable and accurate having repeatability that can be used in resource poor setting to confirm CF diagnosis.

Though sweat chloride remains a biomarker of CFTR-activity as Accurso et al commented,[27] in Bangladesh, methods of sweat collection/estimation spars largely: only 2 CF cases, who were subjected to sweat-collected in increased room temperature as the children were wrapped using aluminum foil to perform bio-chemical analysis on syringe-sucked sweat.[24]

Indigenously Wrapped Sweating Technique

Being enthusiastic from Karba's locally-made technique[26] we also developed our home made locally adoptable, fast, cheap yet effective a technique for sweat collection (Indigenously Wrapped Sweating Technique). Our technique has certain obvious benefits over N. Nahar's one[24] in terms of material used, we wrapped CF-children using plain thick polythene (also easily available and cheap) instead of aluminum foil that they used. Aluminium foil, not easily available, may cause chemical rash/allergy on child's skin but polythene does not. Our method of estimating sweat chloride also differed to their one: we measured sweat-Cl_2 using a modern pre-standardized automated microprocessor-controlled analyzer, plausibly first time in Bangladesh.

Notably, our technique remains inexpensive, affordable, user friendly, acceptable & reproducible. It remain comparable with that of Kabra et al, being appropriate for resource constraint poor-setting communities-which is the strength of our idea/technique as Kabra et al pointed out rightly.[26] Meanwhile, contrary to N. Nahar's method tested on 2 samples of CF-cases, ours one was constituted with adequate number of children. Although we used increased room temperature similar to idea and utility of Nahar et al. to aid in collecting enough sweat to get adequate sweat-Cl_2. An international consensus statement suggests in individuals presenting with clinical features consistent with CF, or a positive family history, a diagnosis of CF can be made if the sweat chloride value is $\geq$60 mmol/L.[19]

Sweat chloride test is repeated only when the value is expressed in intermediate range between 40 and 59 mmol/L.[9,19,20] and we found 9 cases in this group (40-59 mmol/L) and on repetition, 4 cases became positive

(value ≥60 mmol/L). At the initial stage, we referred 5 patients abroad to AIIMS (Professor SK Kabra), New Delhi, India, who could afford and sweat chloride tests for external validity and better patient compliance. In 2 cases the level of sweat chloride were higher from that of our indigenous method and in other 2 cases the levels were low than that of ours (but above 60 mmol/L). However, in one case the sweat level was 37 mmol/L in AIIMS but it was genetically positive. Thus, the overall results of Indian laboratory/diagnosis were quite similar to that of ours evidencing the accuracy and reliability of our results, as correct. On each occasion, we considered as confirmed diagnosis of CF, with a robust support by cardinal clinical features. The diagnosis of cystic fibrosis is supported by the relevant clinical features (respiratory and GI tract), poor nutritional status, steatorrhea, chest radiology/imaging, and sinusitis.

Clinical Manifestations: As Our Findings Attest Typically

Intuitively, we tried to add diversification in knowing all symptoms of childhood CF, more easily. We grouped to categorize all predominant symptoms, arbitrarily, into 3 groups: (i) *"Major Triad of symptoms"* (ii) *"Minor Triad of signs"* and (iii) *Minor duet from history* essentially based on type-specific prominence and rate of presenting symptoms/signs along with past history of illness. Based on these 3 criteria we have provided the diagnostic algorithm on page 16 under the section of "Excerpted Insights from Principal Findings". By *"Major Triad symptoms"* we meant 3 most common yet pertinent symptoms like, persistent cough, respiratory distress and purulent sputum which accounted in –88% of cases on an average. "Persistent cough" prevailed universally among all the 95 (100%) cases, while respiratory distress were reported in >90% cases, followed by purulent sputum 73.7% cases. Similarly, by *"Minor Triad of signs"* we meant other 3 signs like crepitation (83.1%), clubbing (77.9%) and chest in-drawing (58.7%) which accounted in –73% of cases. Important past illness comprising of *Minor duet* of persistent/recurrent pneumonia (83.2%) and bronchiolitis (57.9%), on an average accounted in –70%. While taking history and examining patients of CF, considerations of these important signs/symptoms would help greatly to suspect a CF-case.

Though McCormick observed that majority of CF should had a history of passing bulky fatty stools,[10] we found h/o malabsorption in 37% cases and fecal fat in stool of 53% children. Reasons of this low proportions may be due to the fact that the CF cases having mostly the respiratory symptoms who used to consult with us at the pediatric respiratory group of physicians. We may also speculate that the rates of pancreatic insufficiency leading to fecal fat stool might be less in the Bangladeshi population than in US and European populations due to different genetic variants.

The observation of high proportion of severely underweight, overall wasted stunted under-5 children treated for CF, is consistent with previous studies by Kabra et al.[11] Shah et al.[28] and Aziz et al[11]

There was evidence of bronchiectasis in 60% patients in CXR/ HRCT of chest. The result is consistent with a study done by Aziz.[15]

However, x-ray of paranasal sinuses (PNS) performed among 45 cases (–47%). Of these, 40 CF-cases (89%) were positive for pan-sinusitis, showing a highly significantly association with child's age at diagnosis, older age being more exposed to pan-sinusitis. This observation of high prevalence of sinusitis among children treated for CF is also consistent with the previous studies.[29,30]

A total of 48 patients were evaluated with sputum culture and 13 had bacterial isolates. *Pseudomonas aeruginosa* was isolated from sputum of 17% CF-children though *Pseudomonas spp.* used to be recognized to a good indicator for CF positivity.[23] Interestingly, all the positive sputum C/S revealed growth of *P. aeruginosa*, except in 2 cases which had co-infections with *P. aeruginosa* and *Staphylococcus aureus*. However, *S aureus* in sputum were also reportedly found in CF case.[31] However, in 49% cases (47 children) the caregivers either could not afford the cost of performing C/S or might not have complied to our advice, though very unlikely. *Pseudomonas* was from tracheal aspirate of CF patients in 50% cases by Kabra et al,[11] 28% cases by Aziz et al[15] and in 28% cases by Shah et al.[28] These findings remain higher than that of in our cases, the potential reasons are: many of our microbiological laboratories are not so standard and well equipped, so the quality of bacterial growth might differ from case to case. Moreover, we cultured bacterial growth from sputum whereas others from tracheal aspirate that has more chances of yielding bacterial growth, *per se*.

Socio-Demographic Profile and Pathognomonic Characteristics of CF-as Our Data Explains

Age distribution of our CF-affected children remain consistent or comparable with some other reports though not similar. No significant association was found between age at diagnosis with sex ($P > .10$), which also did not show any significant association religion, since most were Muslims (93%), ($P > .6$). Similar reports were seen from Pakistan,[15,28] Egypt,[16] Iran.[23]

Socio-economic status remains another important factor to have CF as several reports evidenced. Thus,

socio-economic status of most children was low: 56 (59%) belonged to lower 27 (28.4%) to -mid income groups who used to maintain their livelihood with a very tight monthly budget (on average) of <26 679 Bangladeshi Taka/currency (Approx. 315 US dollars). Poor socio-economic status was associated with CF from developing countries from India,[32] Pakistan,[15] Iran[23] Egypt.[16]

Consanguinity and Its Association with CF Cases as a Socio-Religious Factor

Consanguinity (mostly prevalent in Muslim families)[33] remain one of the important covariates for having CF as reported by several authors. Our study revealed consanguinity in 22% of child's family. This remains at a much lower compared to that of from some other Muslim countries, who remains the worst sufferers. In 2017, Aziz et al. from Pakistan reported about 50% CF-cases occurred among children whose parents had consanguineous marriage,[15] and Farahm et al. reported it by 68% from Iran in 2013.[34] However these figures clearly contradict with that of a report by Kabra et al. from India showing it by only 19%[11] which is even less than that of ours (22%).

Factors of Delayed Diagnosis-Our Experience as Non-Caucasian

Our data yielded a time lag of >73 months due to significant difference between age at diagnosis (mean 90.0 ± 48.5 months) than age of disease onset (mean 16.9 ± 26.6), ($P < .01$).This time lag was experienced by several authors.[2,11,14] However, this delay may be in either ways: either the parental unwillingness to see a physician due to financial constraint or travel restraints; on the other hand, this might also happen that the physician/pediatrician himself might have missed the diagnosis of CF. About 17% children were given anti-tubercular therapy presuming to be suffering from PTB without having a strong evidence (Table 2). Nevertheless, noncompliance of doctor's advice due to parental lack of awareness cannot be over ruled, too. Interestingly, a recent report shows that delayed diagnosis are more common in non-Caucasian[5] ethnic minorities,[35] Indians[14] while Gaskin et al[36] warns that non-Caucasian patients with pancreatic sufficiency remain more vulnerable to delayed diagnosis-a phenomenon, that we had been keen to observe readily in case of all our CF-children.

However, few reports from Indo-Pak sub-continent contradict our findings of delayed diagnosis being much less than those. In 2003, Karba et al observed a mean delay of 54 months in Indian patients[14] whereas just recently, in 2017, Aziz et al reported it at even lower (36 months) in Pakistan patients.[15] Further, mean age of onset (symptoms) in our cases was 16.9 ± 26.6 months being similar to both of Kabra et al[14] and Aziz et al.[15] Several reports hinted that such wider gap between onset and diagnosis basically indicates low suspicion index[14] compounded by lack of awareness on CF-cases in Bangladeshi children in adjunct to unavailability of proper diagnostic facilities to facilitate early diagnosis of CF.

During the course of treatment of large cohort of our study patients with CF, we observed 11 deaths. Of them, 10 died of respiratory failure and one from GIT-bleeding. Although, we had lack of multi-disciplinary approaches in treating CF diagnosed with our simple low cost technique, increased effort of continued long term follow-ups with structured questionnaire may help to increase life expectancy and thereby reduce early deaths especially in resource poor setting.

Limitations of the Study

Alike most of reported studies, our study also had certain limitation mostly in terms of inadequacy of logistics and diagnostic methods. We had to conduct this study despite lot of constraints. The standard sweat chloride test could not be established during the long-term time-lag between ages of disease onset and diagnosis. Genetic analysis[19] is also a part of diagnosis of CF and we could not utilize this advantage at all because of 2 prudent reasons: (i) unavailability of genetic tests, and (ii) lower socio-economic condition. Thus, due to lack of genetic confirmation we cannot rule out potential inaccuracies of sweat testing in malnourished children, although there is positive sweat test and CF clinical phenotype.

Also, we could not conduct lung function tests on the CF cases because of lack of organized pediatric respiratory laboratory. We only could do FOB on only 2 patients of CF cases for studying BAL for microorganisms. The microbiology laboratory support was not up to the mark of standard level to yield organisms in every lab.

We still also lack in newborn screening for CF. We could not afford to do the Nasal Potential Difference[37] test which can detect classic and non-classic form of cystic fibrosis. Because of lower socioeconomic status, the regular follow up could not be maintained by the parents with their children of CF particularly coming from far flung areas.

Excerpted Insights from Principal Findings

Excerpted Insight #	Principal findings directing major policy implications	Key points
1.	Cystic fibrosis (CF) exists in Bangladesh far more than anticipated	CF among Bangladeshi children demonstrated longer mean lag between age of onset & disgnosis
2.	A quiet a long mean lag (7.5 yrs) between "age of onset" and "age at diagnosis"	
3.	Longer time lag evidences of lower suspicion index and lack of diagnostic facilities	
4.	Pulmonary syndromes of major triad symptoms, minor triad signs and minor duet from history attested by radiological findings along with gross malnutrition- a good algorithm to suspect CF	*Major triad symptoms*: Persistent cough, respiratory distress, purulent sputum *Minor triad signs*: Crepitation, clubbing, chest in-drawing *Minor duet*: Past illness of pneumonia and bronchiolitis
5.	Our locally developed fast, cheap and effective "Indigenously wrapped sweating technique" worked well to collect sweat and measure chloride level using automated electrolyte analyzer	First reported- indigenously wrapped-sweating technique; -sweet analysis done using automated electrolyte analyzer.
6.	Our first-hand data that this study generated will assist physicians in acquiescing clearer and increased concepts on childhood CF incidence in Bangladesh, that might be true to other countries as well	Our first-hand data on childhood CF
7.	Our findings will augment in increasing public awareness in making them more alert on childhood- CF	Findings claim proper public health awareness on CF managemnt

Conclusion

- We presume that CF-cases remain far more in Bangladeshi children than expected.
- Many cases are misdiagnosed due to low index of suspicion and masking by other diseases.
- The clinical features of CF include persistent cough, productive sputum, persistent or recurrent pneumonia, very poor weight gain & bronchiectasis on chest radiography-imaging.

Recommendation

The first priority should be establishing a reliable diagnosis for "classical" CF from pulmonary syndromes of major triad symptoms, minor triad signs and minor duet from history attested by radiological findings along with gross malnutrition. Moreover, this should be supported through establishing a reliable database both at the institutional and national level. It is also essential to develop multi-disciplinary care and counseling for these ill-fated children with CF, as observed in developed countries yielding clear difference in its outcome.

Author Contributions

ARMLK, SR, RBH, KSA, MAHM, RA, AAM, JUM, MDH, NH, SA, and MJC conceived and designed the study; ARMLK and SR collected, merged, and cleaned the data; ARMLK, SR, RBH, KSA, MAHM, RA, AAM, JUM, MDH, NH, SA, and MJC analyzed and interpreted the data; ARMLK, AHM, RA, and KSA gave technical support and conceptual advice; ARMLK wrote the first draft of the manuscript and all the authors reviewed the manuscript; All authors read and approved the final manuscript.

References

1. Riordan JR, Rommens JM, Kerem B. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science*. 1989;245:1066-1073.
2. Zvereff VV, Faruki H, Edwards M, Friedman KJ. Cystic fibrosis carrier screening in a North American population. *Genet Med*. 2013;16(7):539-546.
3. WHO genes and human disease. *Woo Int*. Published December 7, 2010. Accessed January 23, 2013.
4. Sharma GB. Cystic Fibrosis. http//:emedicine.com. Updated March 1, 2010. Accessed on July 28, 2010.
5. Mirtajani SB, Farnia P, Hassanzad M, Ghanavi J, Farnia P, Velayati AA. Geographical distribution of cystic fibrosis: the past 70 years of data analysis. *Biomed Biotechnol Res J*. 2017;1:105-112.
6. Cystic Fibrosis Foundation Patient Registry. *2014 Annual Data Report*. Bethesda, MD: Cystic Fibrosis Foundation; 2015.
7. Chmiel JF, Berger M, Konstan MW. The role of inflammation in the pathophysiology of CF lung disease. *Clin Rev Allergy Immunol*. 2002;23:5-27.
8. Alghisi F, Angioni A, Tomaiuolo AC, et al. Diagnosis of atypical CF: a case-report to reflect. *J Cyst Fibrosis*. 2008;7(4):292-294.
9. Farrell PM, Rosenstein BJ, White TB, et al. Guidelines for diagnosis of cystic fibrosis in newborns through older adults: cystic fibrosis foundation consensus report. *J Pediatr*. 2008;153:4-14.
10. McCormick J, Green MW, Mehta G, Culross F, Mehta A. Demographics of the UK cystic fibrosis population: implications for neonatal screening. *Eur J Hum Genet*. 2002;10(10):583-590.
11. Kabra SK, Kabra M, Lodha R, et al. Clinical profile and frequency of delta f508 mutation in Indian children with cystic fibrosis. *Indian Pediatr*. 2003;40(7):612-619.
12. Powers CA, Potter EM, Wessel HU, Liyod-Still JD. Cystic fibrosis in Asian Indians. *Arch Pediatr Adolecs Med*. 1996;150:554-555.
13. Kabir AR, Mollah AH, Anwar KS, Roy S, Ali A. KAP study among Bangladeshi doctors on childhood cystic fibrosis. Unpublished data. Ad-din Hospital, Dhaka, September, 2018.
14. Kabra SK, Kabra M, Lodha R, Shastri S. Cystic fibrosis in India. *Pediatr Pulmonol*. 2007;42(12):1087-1094.
15. Aziz DA, Billoo GA, Qureshi A, Khalid M, Salman Kirmani S. Clinical and laboratory profile of children with Cystic Fibrosis: experience of a tertiary care center in Pakistan. *Pak J Med Sci*. 2017;33(3):554-559.
16. Falaki MM, Shahin WA, El- Basha NR, Ali AA, Mehaney DA, El-Ahar MM. Profile of cystic fibrosis in a single referral center in Egypt. *J of Adv Res*. 2014;5:563-568.
17. Ahsan MR, Kamrulalam HS, Hasan AR, Islam MZ, Hossain A. Cystic fibrosis–an update. *Bangladesh J Child Health*. 2016;40(3):174-178.
18. Rosenstein BJ, Cutting GR. The diagnosis of cystic fibrosis: a consensus statement. Cystic fibrosis foundation consensus panel. *J Pediatr*. 1998;132(4):589-595.
19. Farrell PM, White TB, Ren CL, et al. Diagnosis of cystic fibrosis: consensus guideline from the cystic fibrosis foundation. *J Pediatr*.2017;181:4-15.
20. Wallis C. Diagnosis and presentation of cystic fibrosis: In: Wilmott RW, Boat TF, Bush A, Chernick V, Deterding RR, Ratjen F, eds. *Kendig and Chernick's Disorders of the Respiratory Tract in Children*. Elsevier: Saunders, Philadelphia, 8th ed.; 2012:763-769.
21. Gibson LE, Cooke RE. A test for concentration of electrolytes in sweat in cystic fibrosis of the pancreas utilizing pilocarpine by iontophoresis. *Pediatrics*. 1959;23(3):545-549.
22. Kabir AR, Haque N, Majumder JU, Akter S, Hossain D. Sweat test in children with chronic sino-pulmonary problems, 2008 Research Compendium, page 156; Prof ARM Luthful Kabir 2017. www.drluthfulkabir.com
23. Pignatti P, Balestrino A, Herr C. Tracheostomy and related host-pathogen interaction are associated with airway inflammation as characterized by tracheal aspirate analysis. *Respir Med*. 2009;103(2):201-208.
24. Nahar N, Begum F, Sultana J, Rahman M, Ahmed AU, Razzaque AK. Cystic fibrosis: Report on two cases diagnosed by using improvised technique of sweat collection and review of literature. *Chest and Heart J*. 2005;29(I):65-70.
25. Sarker PK, Kabir AR. Cystic fibrosis: a deadly disease and the vast majority are unaware of it. *Bangladesh J Child Health*. 2018;42(3):105-107.
26. Kabra SK, Kabra M, Gera S, et al. An indigenously developed method for sweat collection and estimation of chloride for diagnosis of cystic fibrosis. *Ind Pediatr*. 2002;39(11):1039-43.
27. Accurso FJ, Van Goor F, Zha J, et al. Sweat chloride as a biomarker of CFTR activity: proof of concept and ivacaftor clinical trial data. *J Cyst Fibros*. 2014;13(2):139-147.
28. Shah U, Moatter T, Bhutta ZA. Profile and factors determining outcome in a cohort of cystic fibrosis patients seen at the Aga Khan University Hospital, Karachi, Pakistan. *J Trop Pediatrics*. 2006;52:132-135.
29. Morlacchi LC, Greer M, Tudorache I, et al. The burden of sinus disease in cystic fibrosis lung transplant recipients. *Transpl Infect Dis*. 2018;20:e12924.
30. Passarelli Mantovani R, Sandri A, Boaretti M, et al. Longitudinal monitoring of sinonasal and oral bacterial reservoirs to prevent chronic lung infection in people with cystic fibrosis. *ERJ Open Res*. 2020;6:00115-2020.
31. Dasenbrook EC, Checkley W, Merlo CA. Association between respiratory tract methicillin-resistant *Staphylococcus aureus* and survival in cystic fibrosis. *JAMA*. 2010;303(23):2386-2392.
32. Yadav K, Singh M, Angurana SK, et al. Evaluation of micronutrient profile of North Indian children with cystic fibrosis: a case—control study. *Pediatr Res*. 2014;75:762-766.
33. Shamsad S. Prevalence of consanguinity in Muslim community-a review. *Int J Sci Res (IJSR)*. 2015;4(5):2203-2207.

34. Farahmand F, Khalili M, Shahbaznejad L, et al. Clinical presentation of cystic fibrosis at the time of diagnosis: a multicenter study in a region without newborn screening. *Turk J Gastroenterol*. 2013;24(6):541-546.

35. Spencer DA, Venkataraman M, Weller PH. Delayed diagnosis of cystic fibrosis in children from ethnic minorities. *Lancet*. 1993;342(8865):238.

36. Gaskin K, Gurwitz D, Durie P, et al. Improved respiratory prognosis in patients with cystic fibrosis with normal fat absorption. *J Pediatr*. 1982;100(6):857-862.

37. Taylor CJ, Hardcastle J, Southern KW. Physiological measurements confirming the diagnosis of cystic fibrosis: the sweat test and measurements of trans epithelial potential difference. *Paediatr Respir Rev*. 2009;10(4):220-226.