CLINICAL AND GENETIC RISK FACTORS FOR CYSTIC FIBROSIS-RELATED LIVER DISEASE IN EGYPTIAN CF CHILDREN: A SINGLE-CENTER EXPERIENCE

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Abstract

Background: Cystic fibrosis (CF) is an autosomal recessive disease affecting multiple organ systems, including the liver, leading to cystic fibrosis-related liver disease (CFLD). It was noted that CFLD in Egyptian children with CF is more common than in non-Egyptian people with CF (pwCF). The present study aimed to determine the incidence of CFLD and the potential risk factors for developing CFLD in Egyptian children. The correlation between CFLD and the various genotypes prevalent in Egyptian CF children and the comparison of CFLD in Egyptian and non-Egyptian CF patients will be done. **Methods:** The current cross-sectional study included 50 CF cases from Ain Sham University's Pediatric Pulmonology Clinic in Children's Hospital, Cairo, Egypt. The sweat chloride test and genetic studies were done at the time of diagnosis. All patients' caregivers provided informed consent. Additionally, all subjects underwent detailed history taking, laboratory investigations, clinical assessment, and pelviabdominal ultrasound for evaluation of hepatic involvement. **Results:** Male sex, severe genetic mutation (class I and II), long duration, and early onset of the disease were independent risk factors for CFLD development. In addition, pancreatic insufficiency, as well as meconium ileus history, were predictors of CFLD. Diabetes mellitus and severe lung disease were proven to significantly elevate the risk of CFLD development. **Conclusion:** CFLD is not uncommon in Egyptian CF patients as one-third of the patients were found to have liver affection. CF patients with multiple risk factors are at increased risk of developing liver disease.

Introduction

CF is a systemic disease affecting multi-organs. As a result of progress in the treatment of CF, life expectancy has been steadily increasing, leading to increased recognition of other organ involvement, including CFLD.[1] CFLD is triggered by CFTR gene mutation, which is expressed in cholangiocytes and regulates the exchange of transmembrane chlorides, resulting in a modification in the alkalinity as well as hydration of the bile composition.[2] Even though CF often impacts the airways, nearly 10-15 percent of pwCF have CFLD to some degree.[3] In young adults as well as children, the imaging (ultrasonography), clinical or biochemical CFLD prevalence ranges from 2% to 37%.[4]

CFLD is now estimated to be the third most common cause of mortality among CF cases, following organ transplantation complications as well as lung disease. CFLD clinical picture may vary, from mild asymptomatic hypertransaminasemia to cirrhosis.

The pathomechanism of CFLD is related to alternations in bile flow as well as retention and composition of toxins and hydrophobic bile acids damaging the biliary epithelium. The clinical progression of this impairment is gradual but progressive .[5]

In infants, CFLD can be in the form of cholestasis which can be associated with parenteral nutrition and meconium ileus.[6] Although CFLD early diagnosis is critical since clinical symptoms manifest only after hepatobiliary system damage has already been in progress.[7] Nevertheless, diagnosis is difficult owing to the usual asymptomatic presentation as well as the lack of specific diagnostic tools.[8]

It has been speculated that the incidence of CFLD in Egyptian CF cases is more elevated compared to the Western World, causes leading to this difference have not been clear. This study aimed to identify the incidence of CFLD, a predictive factor in Egyptian CF children and compare this to other pwCF globally.

Methods

The current cross-sectional study included 50 CF cases from the Children's Hospital's Pediatric Pulmonology Clinic, Ain Shams University, Cairo, Egypt, and was approved by the hospital ethics committee. Patients' guardians/parents provided informed consent prior to starting the study.

Patients [?]16 years of age were diagnosed with CF through a positive sweat chloride test, and/ or two diseases resulting in the mutation of CF transmembrane conductance regulator (CFTR) were enrolled in this study. Patients were excluded if they have been on hepatotoxic drugs as antifungals and anti-tuberculous drugs; or having associated liver disease (e.g., Chronic viral hepatitis).

The following were collected from each patient: medical history including age, sex and age at diagnosis, past medical history, family history and socioeconomic histories, clinical assessment of pulmonary, hepatobiliary, and gastrointestinal symptoms, laboratory testing including complete blood count (neutrophils, lymphocytes, white blood cell count, hemoglobin, and platelets), liver enzymes as ALT, AST, and albumin, coagulation parameters (PT, PTT, INR). In addition, pelvic-abdominal ultrasound was done for evaluation of the size and echogenicity of the liver, size of the spleen, presence or absence of ascites, portal vein, common bile duct, and gall bladder sludge or stones. Observation of a bright echo pattern of the liver on ultrasound is commonly considered a sign of hepatic steatosis.

A full clinical examination was done including vital signs, weight, height, as well as body mass index (BMI), chest and abdominal examination. Shwachman-Kulczycki score was utilized for assessing CF disease severity based on four different parameters: radiological findings, nutrition status, physical examination, and general activity. Each characteristic is assigned a value between 5 (severely impaired) and 25 (normal), resulting in a total score that is classified as excellent (86-100), good (71-85), mild (56-70), moderate (41-55) or severe (<40).[9]

Statistical Analysis

Data collection, revision, coding, and entry were done utilizing the 23$^{\text{rd}}$ version of the (IBM$^{(\text{R})}$ SPSS). Quantitative data were expressed as ranges, standard deviations, as well as mean, standard deviations, and ranges, whereas qualitative variables were expressed as percentages and numbers.

Comparing groups in terms of qualitative data was performed utilizing the Fisher exact test or Chi-square test. The Independent t-test was used for parametric distribution, while the Mann-Whitney test was used for nonparametric distribution. Multivariate logistic regression analysis was utilized for the strength of the association between risk factors for CFLD. Moreover, the confidence interval was determined at 95 percent, while the accepted error margin was at 5 percent. The significance level was determined at p-value < 0.05 and considered highly significant if $p < 0.01$.

Results

This study included 50 cases, sweat test was 73-159 mmol/L, 80% of the patients had productive cough, 46% had dyspnea, 90% had steatorrhea, 20% had CFRD and 62% of patients had BMI at the <3$^{\text{rd}}$%. There was a positive family history of CF in 32% of the total patient population (16 patients). Nineteen patients (38%) out of the 50 patients were diagnosed with CFLD (Table 1). Seven out 19 (37%) showed liver abnormality through abnormal biochemical labs and ultrasound abnormalities while 12 (63%) had clinical abnormalities, in addition. Compared the CFLD cohort (19 patients) to the non-CFLD cohort (31 patients),

Liver involvement was significantly correlated with male sex, early onset of disease, longer duration of illness, and higher mortality rate with p-value$<$ 0.05, while there was no substantial difference between both groups with respect to consanguinity with p-value 0.34. It was noted that sweat chloride test levels were higher in the CFLD group (Table 1).

There were differences in genetic mutations between both cohorts, with class I and II mutations more frequent in the CFLD cohort with a p-value $<$0.05, while class V mutation was more prevalent in the non-CFLD cohort with a p-value $<$0.05. F508del homozygous was the most common mutation in both groups representing 64.3% of the CFLD cases and 33.3% of the non- CFLD cases (Table 2).

Table 3 shows a statistically significant correlation between CFLD and gender, duration of illness $>$ 5 years, weight and height $<$ 5$^{\text{th}}$ percentile, early onset of the disease [?] 9 months, clinically severe disease, class I or II mutations, non-compliance to pancreatic enzyme replacement therapy as well as fat-soluble vitamins while there was no statistically significant association with Neonatal Intensive Care Unit (NICU) admission due to meconium ileus.

Using Shwachman- Kulczycki score, there was significantly severe disease involvement in CFLD cases (63.2%) while in non-CFLD cases the degree of severe disease was only 16.1% with P-value $<$ 0.01. Most non-CFLD cases presented with mild to moderate disease.

Discussion

The improved CF management in high income countries has led to an increased life expectancy in CF cases. Consequently, as pwCF get older, a substantial liver dysfunction emerges, which complicates the disease with incidence of 10-15%.[10] The occurance of CF in a prospective study was found to be 2.5 per 100 patient-years during early life years, with an increase in the second decade.[11]Hepatobiliary involvement was reported in 4-10.9 percent of Middle Eastern CF cases.[12,13] In a previous study of CF cases in Egypt, the CFLD rate was found to be more elevated compared to the US population.[12] In the current study, the frequency of liver disease in CF patients was 38%, significantly higher than previously reported in the Middle Eastern population. This study revealed that the male sex is a significant risk factor associated with CFLD, which was reported previously in 2 case-control studies in several western countries.[13,14]

A large cohort study was done on 177 pwCF (aged between one month and 23 years) and found that liver disease occurred before puberty in all female patients, and the incidence increased up to 18% by the age of eleven years, but in male patients, the incidence increased from 25% by the age of eleven up to 42% by the age of eighteen.[11]That could be due to endocrine factors involved in CFLD development with the estrogen and its receptors substantially contributing to the modulation of secretory and proliferative activities of the intrahepatic biliary epithelium due to liver damage.[11] This incidence was similar to what we found in our study and it was significantly higher than previously reported in other studies.

In a retrospective analysis of data from 561 pwCF in Canada, Stonebraker et al. found that mutations, as well as male gender related to severe phenotype, increased the progression of severe liver disease.[15] This study had illustrated risk factors including male sex, and class I-III mutations, which is related to meconium ileus as well as pancreatic insufficiency at birth.[15] In a prospective study, F508del was detected in 51-55 percent of CFLD cases.[11] That was similar to what was found in our study where 64% of the patients with CFLD had F508del and that was statistically significant compared to patients that didn't have CFLD (33%).

In addition, the young age at diagnosis and the longer duration of the disease, the more susceptibility to CFLD development. This is in agreement with a study, in which the median age of onset of CFLD was between 6-8 months.[16] In a study regarding risk factors leading to CFLD in a large-scale cohort of patients in France concluded that male sex and long duration of illness are significant risk factors for developing CFLD, they also found that CFLD occurrence elevated by 1-2 percent every year from birth until the age of 25 years of age.[17] Also, Fagundes et al. and Efrati et al. in a study including one hundred and fifty pwCF between 1975 and 2000 in the National Cystic Fibrosis Center in Israel revealed that the early onset of CF and long duration are significant risk factors for developing CFLD.[18,19]

CFLD often manifests during the two decades of life. The majority of CF children tend to develop a degree of steatosis. CF-related multi-lobular cirrhosis or focal biliary cirrhosis occur in children and adolescent, but major biliary involvement resembling sclerosing cholangitis is more common in adults. In severe CFLD, portal hypertension, particularly non-cirrhotic portal hypertension, might cause variceal bleeding.[20]

It has been reported that severe CFLD is life-threatening because it is associated with the progression of portal hypertension, and worsening of the respiratory status and liver transplantation might be indicated.[21] In our CFLD patients, 89% of the patients had productive cough and 46% had dyspnea. Steatorrhea was present in 90% and CF-assocaited diabetes CFRD was present in 37% of patients. In addition to 68% of patients had hepatomegaly, 16% had splenomegaly, 57% had abdominal distention, 2 patients had gall bladder stones and 1 had ascites. None of the patients had portal hypertension, biliary dilation, or jaundice. In accordance with these findings, Colombo et al. observed hepatomegaly in 6–30% of cases,[21] even though our patients had higher rate of hepatomegaly (68%).

In our patients with CFLD, abdominal ultrasound revealed hepatomegaly in 28%, hepatic steatosis in 24%, Cholelithiasis in 4% (males), ascites in 2%, and splenomegaly in 16%. That was similar to the finding in a study, which revealed hepatic steatosis in 20–60% and Cholelithiasis was in 1–10% of the studied patients in a study including a group of 124 children with an average age of 5.4 years in the United Kingdom.[22] A retrospective review of sequential abdominal ultrasound reported that 11.9% of the patients had splenomegaly, seven cases (4.2%) had frank cirrhotic changes on ultrasound criteria, and eight cases (4.8%) experienced gall bladder stones with male predominance in this group (6/8).[23]

History of meconium ileus was reported in the CFLD cases representing 26.3% than the non-CFLD cases representing 6.5%. While Bock et al. revealed that meconium ileus was reported in 20% of the cases.[24] Colombo et al. reported that cases with a history of meconium ileus were five times more prone to develop CFLD compared to cases without.[11] The development of liver disease in intestinal obstruction cases could be due to inspissated bowel that leads to biliary secretion that plugs the bile ductule leading to liver damage. One-fourth of patients in this study developed liver disease.

This study showed that CFRD is a major risk factor associated with CFLD development as 37% of patients were diagnosed with CFRD. That was similar to a study that reported, that 32% of patients with CFLD had CFRD.[25] In another study, CFRD was a considerable risk factor for CFLD development in Italian CF Registry.[26]In addition**,** this study showed that pancreatic insufficiency, meconium ileus history, and younger age at diagnosis, were all related to severe CFTR mutation classes, and were proven as risk factors for CFLD development.[26] Another study reported that severe mutations (classes I, II, or III) manifesting with elevated mortality as well as morbidity are substantially related to younger age (<1 year), meconium ileus, as well as pancreatic insufficiency at diagnosis, and liver affection.[27] Another study reported that mild CFTR mutations (class IV and class V) are uncommon in individuals with CFLD.[28] Our study revealed that severe mutations including classes I and II was a significant risk factor associated with the development of CFLD. Furthermore, F508del is the most common mutation representing 64.3% of the studied patients. That was similar to previous reports, of correlation between CFLD and severe genetic mutations (class I and II) in retrospective studies to investigate potential risk factors for developing CFLD in the Netherlands.[11,19,29]

In this study, CFLD was associated with severe CF disease as per Shwachman score (63.2% of the CFLD group had severe CF disease compared to 16.1% in the non-CFLD group). Patients with CFLD had frequent hospital admissions and impaired growth and nutrition. Similarly, it was reported in some studies that, children with established CFLD suffer from impaired nutrition and growth, severe lung disease with worse pulmonary function, as well as altered body composition .[26,30] In another study, similar findings were noted.[13]

Early liver disease diagnosis is a significant problem for the clinical management of those cases. Usually, liver-associated symptoms might be undetectable until cirrhosis and portal hypertension are developed.[21] All our CFLD patients were treated with ursodeoxycholic acid. Nevertheless, Cochrane Reviews have shown that the effectiveness of ursodeoxycholic acid has not been proven clearly.[31]

As there is no efficient medication to treat or inhibit the development of cirrhosis, portal hypertension, or

fibrosis in CFLD, its management should be carried out by a multidisciplinary team and is mostly supportive. Liver transplantation (LT) might be recommended for CFLD cases with intractable complications of portal hypertension or end-stage liver disease since it provides substantial survival advantages. However, LT does not always enhance long-term pulmonary prognosis. Therefore, subjects with liver disease, as well as combined lung disease, may undergo combined lung and liver transplantation (CLLT).[32]

This study revealed in addition, that non-adherence to pancreatic enzyme replacement therapy represent 78.9% of the CFLD group while representing 38.7% of the non- CFLD group. In addition, non-adherence to fat-soluble vitamins represents 47.4% of CFLD group vs. 16.1% in the non-CFLD group. These findings could be factors in developing CFLD, as well.

Conclusion

CFLD is not uncommon in CF as one third of the CF patients were found to have liver involvement in this Egyptian patient population diagnosed clinically, biochemically and/or by ultrasound. CFLD incidence was elevated in male individuals, patients with duration of illness > 5 years, patients with weight and height $<$ 5th percentile, early onset of disease before the age of 9 months, history of meconium ileus, patients not adherent to fat-soluble vitamins as well as pancreatic enzyme replacements, patients with severe CF disease and patients with class I and II genetic mutation. More studies from low- and middle-income countries like Egypt, are needed to help understand the nature of the disease, improve patients' quality of life, improve survival and help improve the disease outcome globally.

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