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Cystic fibrosis liver disease – from diagnosis to risk factors

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Abstract

Cystic fibrosis (CF) is the most frequent monogenic genetic disease, autosomal recessive transmitted, characterized by an impressive clinical polymorphism and appreciative fatal prospective. Liver disease is the second non-pulmonary cause of death in cystic fibrosis, which, with increasing life expectancy, became an important management problem. Predisposing factors like male gender, pancreatic insufficiency, meconium ileus and severe mutation are incriminated to influence the occurrence of cystic fibrosis associated liver disease (CFLD). Our study included 174 patients with CF, monitored in the National Cystic Fibrosis Centre, Timisoara, Romania. They were routinely followed-up by clinical assessment, liver biochemical tests, ultrasound examinations and other methods like transient elastography, biopsy, in selected cases. Sixty-six patients, with median age at diagnosis 4.33 years, diagnosed with CFLD, without significant gender gap. CFLD was frequent in patients aged over eight years, with meconium ileus history, carriers of severe mutations (p=0.002). Pancreatic insufficiency, although present in 75% of patients with CFLD was not confirmed as risk factor, not male gender, in our study. CF children older than eight years, carriers of a severe genotype, with a positive history of meconium ileus, were more likely predisposed to CFLD.

Keywords: cystic fibrosis associated liver disease (CFLD), cystic fibrosis transmembrane regulator (CFTR), mutation, children.

Introduction

Cystic fibrosis (CF) is the most frequent monogenic disease in population with Caucasian origin [1], with autosomal recessive transmission and a significant fatal prospective [2]. The disease's incidence worldwide is about one in 2500–3000 of newborn [1], with a significant mutation portage rate of 1 in 25 people [1]. Genetic substrate consists in mutations in the CFTR (cystic fibrosis transmembrane conductance regulator) gene that encrypts the activity of the chloride conductor protein [2]. Patients with CFTR mutations cystic fibrosis have abnormal chloride conduction across the apical membrane of epithelial cells, resulting in thickened secretions in the respiratory tract, pancreas, liver, intestines, sweat glands, deferens vessels [1]. The clinical picture of cystic fibrosis is impressive, consisting in pulmonary obstructive disease, liver disease, steatorrhea, related diabetes, failure to thrive, etc. [3]. Children with cystic fibrosis associated, until recently, a disappointing life expectancy; in 1938, when the disease was first described, the fatal outcome occurred in early childhood [4]. Nowadays, with all improvements in the disease's management, the life expectation rate is more than 40 years, with an evolution encumbered by multiple complications like hepatic cirrhosis, pulmonary failure, diabetes, osteoporosis [5]. Cystic fibrosis associated liver disease (CFLD) is the most important non-pulmonary cause of death, being responsible of 2.5% of patients [6]. CFLD pathogenesis is secondary to the CFTR protein defect in the cholangiocytes; the effect is an increase viscous secretion and subsequent biliary obstruction, leading to focal biliary cirrhosis and progressive periportal fibrosis [2, 7]. Although the disease has a monogenic substrate, not all the patients develop CFLD [6, 8], while in some cases the liver involvement represents the main management issue [8, 9]. The pathogenesis is apparently multifactorial, with contributions from genetic determinants and other factors like environment. Risk factors like pancreatic insufficiency (PI) [6–8], male gender [6], meconium ileus (MI) [6, 10] or severe mutation [8, 9, 11] failed to demonstrate a direct relation in every patients [12]. It seems that other factors, as antioxidants deficiency, drug's hepatotoxicity, environmental features and genetic modifiers could influence CFLD evolution [13]. The life expectancy and disease's evolution could be improved by early diagnosis and an accurate consequent management [14–16].

The aim of the study was to evaluate the presence of CFLD in our Romanian patients and the relations with suggested risk factors like male gender, pancreatic insufficiency, meconium ileus or severe mutations.

Patients and Methods

One hundred seventy four patients diagnosed with typical form of CF since 2003 to 2013, followed in our National Cystic Fibrosis Centre, Timişoara, Romania aged between 6–24 years were included in the study. Patients or their parents accepted the inclusion in the study by signing the written informed consent, in conformity with Helsinki Declaration. Ethical Committee of the Clinical County Hospital, Timişoara, approved the study developed in National Cystic Fibrosis Centre of Timişoara. During April 2012 to June 2013, all patients were appointed to the National CF Centre for enclosure

in the transversal study. A detailed history regarding age-at-diagnosis, presence of meconium ileus, pancreatic insufficiency, besides physical evaluation, biochemical assessment, ultrasound examination and transient elastography was completed.

Cystic fibrosis associated liver disease (CFLD) was defined by the presence of at least two of the following features:

1. Hepatomegaly ± splenomegaly, clinically detected and ultrasound confirmed,
2. Persistent elevation of liver function test, and
3. Liver parenchyma alteration, detected at ultrasound examination, transient elastography or MRI, according to the current guidelines.

Clinical evaluation included detection of hepatomegaly (liver span >2 cm below the costal border on the medioclavicular line) and splenomegaly (detection of spleen at >1 cm below left costal board). Biochemical liver investigations including dosage of serum transaminases [aspartate aminotransferase (AST), alanine aminotransferase (ALT)], gamma-glutamyl transferase (GGT) and bilirubin fraction were done in the Central Laboratory of Clinical County Hospital, Timişoara, and were taken into consideration only when persistently increased over 1.5 normal values at three determinations.

Genetic tests were performed at the Department of Biochemistry, "Victor Babeş" University of Medicine and Pharmacy, Timişoara, for patients diagnosed after 2002, by a method employed by the Elucigene^{TM} CF29 kit uses ARMS (Amplification Refractory Mutation System) allele specific amplification technology. Genotypes performed before 2003 (in collaboration with Genetic Unit of Manchester University, UK) were collected from Timişoara National CF Center's files, with specific agreement of patients and legal tutors.

Ultrasound (US) examination was performed in the Department of Pediatrics II, "Victor Babeş" University of Medicine and Pharmacy, Timişoara, using a 3.5–5 MHz convex probe with Doppler flow. Ultrasound (US) features were quantified according to the Williams score, stating increased liver echogenicity, heterogeneity, nodularity, irregular margins, signs of portal hypertension.

In selected cases, with inconclusive ultrasound features or increase CFLD gravity, other examinations like: magnetic resonance imaging (MRI), scintigraphy or liver biopsy, were performed. Transient elastography (TE) was used for measurement of liver stiffness by using FibroScan (Echosens, France) device in CFLD patients, through the intercostal space using a cut-off value of liver stiffness of 5 kPa [17].

For statistical processing, IBM-SPSS v. 18 was used. For the description of the continuous variables, we used the mean and the standard deviation and for the description of ordinal and nominal variables, we used frequency and percentage. A chi-square test of independence with Yates continuity correction was used to determine the association between the risk factors and the presence of CFLD. The threshold level used for p-value was 0.05 and the exact value of p was reported. Odd ratio was calculated for risk factors mentioning 95% confidence interval values.

Results

CFLD was diagnosed in 66 of our patients, signifying a cumulative prevalence of 37.9%, comparable with other reported data. Patients were diagnosed with CF at a median age of 4.33 years ± 5.18 SD with a "free" interval of approximately four years until CFLD occurrence.

Median age at CFLD diagnosis was 7.92 years ± 4.54 SD, with a predominance in the patients aged over 12 years, confirming the supposition that liver disease commonly occur after 10 years of evolution. In three children, the liver disease occurred in infancy, being the first manifestation of CF; all this children had an unfavorable evolution, with development of portal hypertension. Severe liver disease (comprising liver cirrhosis) was diagnosed in 12.12% of patients, all pancreatic insufficient. Unfortunately, we lost two patients because of pulmonary status deterioration, with aggravated respiratory infection and pulmonary failure.

Following the present guidelines for CFLD diagnosis, besides clinical evaluation and biochemical assessment, liver parenchyma was evaluated by multiple methods.

On ultrasound, different patterns were found, from increased echogenicity or focal steatosis to heterogeneous parenchyma, characteristic for multilobular cirrhosis. All CFLD patients had ultrasonographic changes consisting in increase echogenicity – diffuse or focal (Figure 1), heterogeneity of liver parenchyma (Figure 2), splenomegaly and signs of portal hypertension.

The ultrasonography's "weak point" is that hyperechogenicity could suggest steatosis or fibrosis, but is extremely difficult to differentiate between these features with a usual ultrasound machine. Here is the role for transient elastography, who evaluate the stiffness of liver parenchyma.

In our study, transient elastography was performed in 30% of patients with CFLD, median value of liver stiffness was 8.96 kPa ± 5.83 SD, significantly increase over the established cut-off value of 5 kPa indicating liver fibrosis. We found a good individual correspondence between ultrasound changes and elasticity index. The fibrosis index was accurately associated with age of patients, the major fibrosis degree of 27.2 kPa being found in a 22-year-old student.

Although is regarded as the "gold standard" in many chronic liver disease, biopsy has a limited role in CFLD diagnosis, because the focal distribution of the lesions in cystic fibrosis liver. Liver biopsy can provide important information on the predominant type of lesion in CFLD: focal biliary cirrhosis, biliary pigment deposits, portal fibrosis, steatosis, biliary ducts proliferation with epithelial multistratification.

In our patients, liver biopsy was performed in two selected cases only when other overlaps unidentified pathologies were suspected. Biliary pigment deposits, neoductular proliferation with the presence of a rim of polymorphonuclear leukocytes and lymphocytes around the bile ducts was found in a 6-month-old girl (Figure 3) and fatty hepatocyte infiltration with portal fibrosis in a 2-year-old toddler (Figure 4).

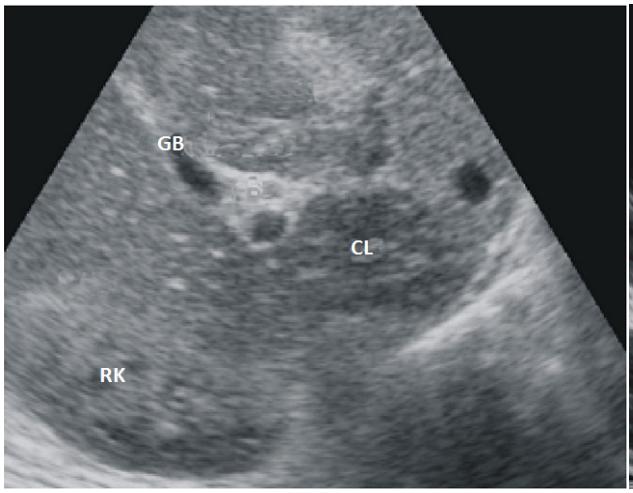

Figure 1 – ***Focal and diffuse hyperechogenicity of hepatic parenchyma, possible steatosis. CL – Caudate lobe, GB – Gallbladder, RK – Right kidney.***

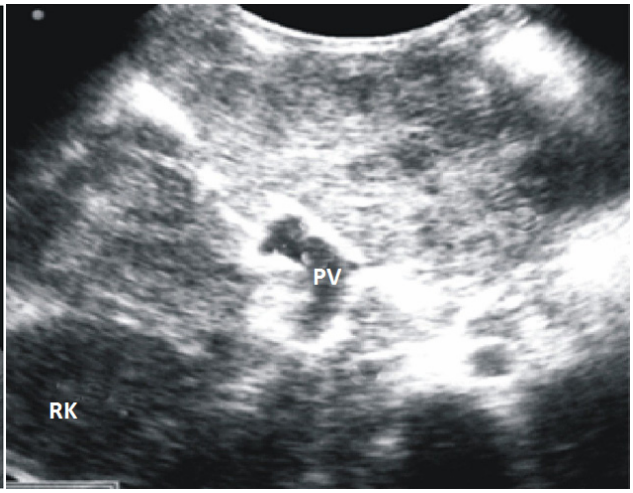

Figure 2 – ***Liver heterogeneous parenchyma, alternation of hypoechoic with hyperechogen texture. PV – Portal vein, RK – Right kidney.***

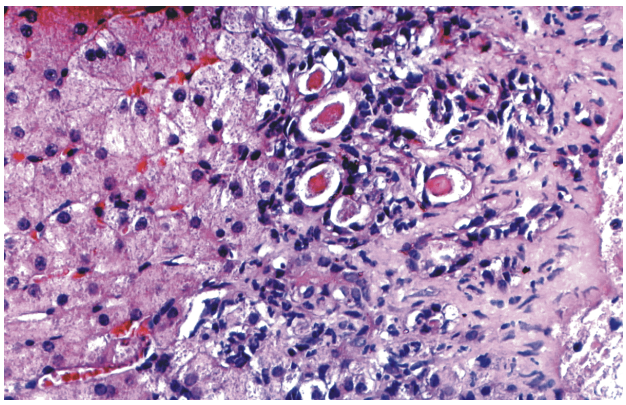

Figure 3 – ***Periportal neoductular proliferation with large plugs of proteinaceous material; pericholangitis. HE staining, ×400.***

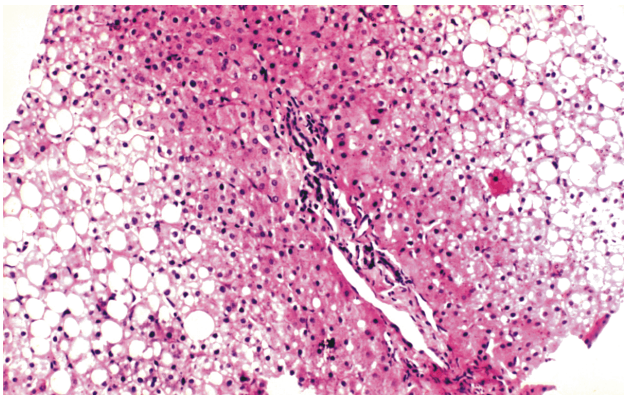

Figure 4 – ***Macrovesicular steatosis: hepatocytes' fatty infiltration. HE staining, ×100.***

The genetic testing of our patients showed an increased percentage of severe genotypes (Figure 5): 44% of children had homozygous F508del genotypes, 9% were heterozygous for F508del/G542X genotype. The other genotypes were equally represented 2%, with an important percent of 6% of unknown genotype. F508del was the most frequent mutation with an appreciate prevalence of 65.9%; second prevalence was attributed to unknown mutation (X), present in 14.4% of CFLD patients. Thirdly as frequency, G542X, also a severe mutation was registered for 5.3% of CFLD patients.

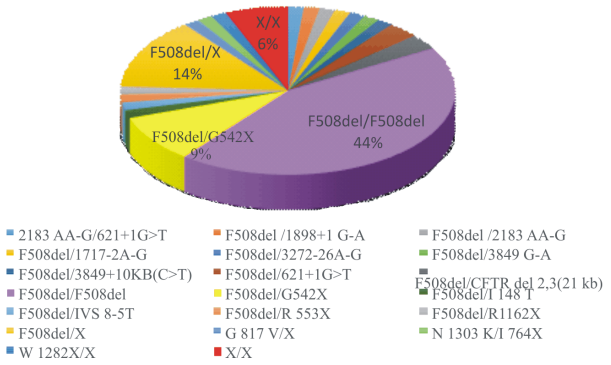

Figure 5 – ***Genotypes' distribution.***

In our study, severe mutations were detected in 78% of CFLD patients. The odds that a carrier of a severe mutation develops CFLD was found to be 4.79 higher (95% CI: 1.8–12.7) signifying that severe mutation associates frequent the development of liver disease in cystic fibrosis. The fact that a severe mutation of class I, II or III is risk factor for CFLD was confirmed by chi-square test of independence with Yates' continuity $\chi^2(1)=9.77$ ($p=0.002$).

Although patients have similar genotype, not all of them develop CFLD. Many factors like male gender, pancreatic insufficiency, meconium ileus and severe mutation were incriminated as risk factors for CFLD development.

Despite that, some studies found that CFLD occur more frequent in male patients, in our study, the distribution of CFLD was approximately equal between genders (slight predominance of female gender of 53%). There were not significant differences of gender distribution between the groups with and without CFLD ($p=0.327$) which show that in our population, male gender was not a risk factor for CFLD development.

Pancreatic insufficiency (PI) was majoritarian in all patients, with a prevalence of 78.73%, being present in almost half of patients with CFLD, and frequent also in CF patient without liver disease. No significant association between PI and CFLD was observed, indicating that not any pancreatic insufficient child with CF will develop CFLD (OR= 0.75; 95% CI: 0.36–1.57).

For evaluation of meconium ileus (MI) as a risk factor, a chi-square test of independence with Yates' continuity correction indicated that CFLD is 2.63 times more often associated with the presence of meconium ileus, $\chi^2(1)= 3.96$ (p=0.046), which confirm the fact that MI is a risk factor in our patients. Only a small percentage of 5.7% CF patients without liver disease had meconium ileus. The presence of meconium ileus in the patient's history was strongly associated with CFLD occurrence in our patients, the risk for a CF patients with MI history was 2.6 much more than in patients without MI (95% CI: 1–6.35).

Discussion

Our study confirms the important prevalence (37.9%) of cystic fibrosis associated liver disease in our patients, similar with other data (27–41%) reported in longitudinal prospective study [6, 9, 11]. The explanation consist in the uniformity of criteria for CFLD diagnosis and the constant evaluation of clinical, biochemical and paraclinical feature specific for CFLD. Cystic fibrosis was diagnosed relative early in our study, at 4.3 years median age, suggesting the increase awareness about this monogenic disease. Although is a frequent pathology and should be diagnosed more often, the diagnosis of CF is delayed in several patients, probably because of clinical polymorphism, which overlap with other pathology [4]. Unfortunately, a delayed diagnosis came with a cluster of installed complications, like liver disease or related diabetes [3].

In our children patients, the liver disease was diagnosed at about eight years of age, with a "free" interval of approximately four years since CF diagnosis. In the majority of patients, CFLD arise during adolescence or puberty, but in a group of children the disease occur with liver failure or severe liver disease, fact described in our study also, where CFLD arose in three infants, being the first expression of cystic fibrosis. Ultrasound Williams scoring system used [18] was helpful for diagnosis and monitoring of CFLD evolution, especially when transient elastography was associated. Transient elastography was extremely helpful in conforming fibrosis on cases with ultrasound detected liver hyperechogenicity, fact establish in other studies [17, 19].

CFLD evolution is variable; in the majority of children, the progression is slow while in some patients, CFLD has a violent evolution, with rapid progression to hepatic failure and death [8, 9, 12]. The reasons of this variability are still unknown and predicting who will develop CFLD is very difficult.

As a monogenic auto recessive disease, genetically express by a combination of two mutations on the form of a genotype, CF was expected to have clinical manifestation according to genotypes [20]. For example, patients with the same genotype would be anticipated to have identical clinical manifestations, which are not happened, not even in siblings [21]. Over the years, many factors were incriminated to predispose to CFLD development. The male gender was one of the supposed risk factor, declared in some studies [6], which was not confirmed by our study. Another clinical feature considered to be frequently associated with CFLD is pancreatic insufficiency [6, 8, 9, 11]. Documented in a large percent (78.73%) among CF children, PI had a low correspondence with liver disease in our patients. The explanation could be the inclusion in the study of typical form of CF patients, who pancreatic insufficiency is mandatory; therefore, even if PI was found in 75% CFLD patients, a similar percentage existed in CF population without liver disease.

Severe mutations, well known associated with PI [5], were confirmed as risk factor for CFLD, in our patients, highlighting the fact that a carrier of severe genotype have a five times more risk to develop CFLD (4.79 odd ratio). The frequency of severe mutation was 78% of CFLD patients and the severe genotypes represented majority in 63% of patients, according to other studies [6, 8, 9, 11, 22] and to the Central European spectrum of mutation [23]. An important share was owned by unknown genotypes (X/X), suggesting that another mutations, not detected by the 29 kit spectrum used in our study, were "responsible" for CFLD occurrence.

Meconium ileus correspondence in CFLD was present, indicating that CFLD is 2.6 times more often associated with the presence of meconium ileus. The presence of meconium ileus in the patient's history was strongly associated with CFLD (p=0.046), which confirm the fact that MI is a risk factor in our patients (2.63 OR; 95% CI: 1–6.35).

In conclusion, several factors have been accepted to be significantly related with CFLD, including meconium ileus, but the exact role as predisposing factor for the development of CFLD remains arguable [8].

Following the present guidelines for CFLD diagnosis [15], besides clinical assessment and history, diagnosis of CFLD require a persistent elevation of liver function tests and modified liver parenchyma, any of this feature, singularly, is not significant for diagnosis. For example, elevation of liver test, not specific for CFLD, could be secondary to drug's hepatotoxicity or viral hepatitis. In the same manner, ultrasound changes like multilobular nodularity indicate cirrhosis, while increased echogenicity is suggestive for fatty infiltration or hepatic fibrosis, but it cannot be differentiated by usual ultrasound techniques [18]. Transient elastography is considered a reliable method for diagnosis of liver fibrosis in other liver diseases [19]; in our study, it prove a good accuracy, with detection of increase liver stiffness (>5 kPa) in several children who presented only increase liver echogenicity on US. The median value of liver stiffness was 8.96 kPa, suggestive for moderate fibrosis, implying that cirrhosis will occur in our patients. In cases with portal hypertension diagnosed on ultrasonography, there was a fair individual correspondence with elasticity index. Although biopsy is a very good method for highlighting lesions existent before the disease it's clinically manifested [24], because of the invasivity and the patchy distribution of lesions it is not routinely reliable [25].

Starting from the fact that it could not demonstrate any definite association with the presence of CFLD and a certain mutation, and taking into account the highly variable phenotype corresponding to an identical genotype, several questions were raised regarding potential influence. It seems that other factors, like genetic modifiers could be responsible for CFLD occurrence [21]. Recently published data suggest that serological biomarkers (TIMP-4, Endoglin) would be reliable non-invasive tools for CFLD diagnosis [26].

Conclusions

Cystic fibrosis liver disease has a multifactorial pathogenesis, with contribution from genetic to an increasing prevalence in Romanian patients. CF children older than eight years, carriers of a severe genotype, with a positive history of meconium ileus, were more likely predisposed to CFLD. For an accurate diagnosis, is necessary that CF patients to be screened annually by clinical assessment, biochemical assessment and ultrasonography examinations. While biopsy is not a commonly endorsed investigation, transient elastography demonstrate an important reliability for fibrosis detection and should be used regularly. Even we could not find a specific mutation correlated with CFLD, the liver disease was more frequent in carriers of severe mutations, suggesting that children harboring severe genotype will develop, with large probability, CFLD.

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