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Liver Involvement in Iranian Children With Cystic Fibrosis: Ultrasonography and Biochemical Findings

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ABSTRACT

Background: Liver disease is increasingly common in pediatric cystic fibrosis (CF). Liver dysfunction in CF patients is an early complication and relatively common which may progress silently.
Objectives: The purpose of this study was to determine the prevalence of abnormal liver architecture by Ultrasonography (US) and their associations to abnormal liver function tests particularly abnormal Gamma Glutamyl Transpeptidase (GGT), Alanine Aminotransferase (ALT), and Aspartate Aminotransferase (AST) level for its early detection before the complications occur.
Patients and Methods: This study as a cross-sectional study was performed at the Children's Medical Center Hospital, Pediatrics Center of Excellence in Tehran, Iran. In all, 114 patients with cystic fibrosis (70 boys, 44 girls) were enrolled. Sample blood test including AST, ALT, GGT and abdominal Sonography was obtained from all patients. Abnormal liver function test was defined by two consecutive occasions; ALT and/or AST levels were ≥ 2 times the upper limit of normal values. GGT normal values were defined by patient age. Data were analyzed using $\chi 2$ test and independent T test. Statistical significance was defined as P values of < 0.05 by SPSS ver.19 software.
Results: Abnormal liver function test was detected in CF patients. As well, liver sonogram was abnormal in approximately one-third of the patients. This study showed a higher prevalence of biochemical abnormality in patients with abnormal livers ultrasonography.
Conclusions: Noninvasive paraclinical evaluation methods could be recommended in the patients with CF for early detection of silent liver abnormalities before progression to end stage liver disease.

Keywords: Cystic Fibrosis; Ultrasonography; Liver Disease; Children

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Implication for health policy/practice/research/medical education:
Screening of Cystic fibrosis patients with Liver Function Tests and Ultrasonography leads to early detection of liver abnormalities. Reading of this manuscript is beneficial for general practitioners, internists, Pediatrics, Radiologists and Gastroenterology specialists.

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1. Background

Cystic fibrosis (CF) is the most common life-threatening genetic disorder in Caucasian children (1). CF is an inherited autosomal recessive disease of epithelial transport which affects most critically the lungs besides pancreas, liver and intestine (2). The disease results from mutation of a gene located in the long arm of chromosome 7 which encodes the CF transmembrane conductance regulator (CFTR) protein (3). CFTR protein is necessary in the regulation of chloride transport in epithelial cells. The defective protein coded by the mutated gene leads to decreased chloride secretion and increased sodium re-absorption (4). In the liver, CFTR protein is located in biliary apical domain of intrahepatic, extra-hepatic and gallbladder epithelia, where it appears to play a role in normal bile formation (5).

Lack of a functional CFTR protein on the biliary epithelium could lead to hyper viscous secretions in the liver, predisposing CF children to liver disorders (6). Liver involvement in patients with CF is an early complication and relatively common which may progress silently (1). With improved survival of CF patients and reduced death from extra- hepatic causes, hepatobiliary involvement is increasingly recognized as a major manifestation of the disease. Since clinical manifestations of liver involvement are usually present in advanced phases, a means of detecting or predicting liver damage particularly at an earlier stage is needed. Ultrasonography (US) is used as one of several tests to evaluate hepatic disorders as hepatosplenomegaly, cirrhosis, and portal hypertension in CF patients. In this regard, a cohort study in CF patients with initial normal liver US revealed liver damage progression after ten years follow-up to cirrhosis and portal hypertension (7). Besides, another study in 195 CF patients has shown that there is a significant relationship between abnormal liver architecture at US and results of LFT in CF children (8). Since liver disease in patients with CF is clinically asymptomatic with lack of a sensitive or specific marker for diagnosing, this study was performed to determine the frequency of abnormal liver architecture by US and its associations with abnormal liver function tests in Iranian CF patients.

2. Objectives

The purpose of this study was to determine the prevalence of abnormal liver architecture by Ultrasonography (US) and its association with abnormal liver function tests particularly abnormal Gamma Glutamyl Transpeptidase (GGT), Alanine Aminotransferase (ALT), and Aspartate Aminotransferase (AST) level for its early detection before the complications occurs.

3. Materials and Methods

3.1. Study Design

This study was cross-sectional study that performed between 2009 and 2011 in the Children's Medical Center, Pediatrics Center of Excellence of Tehran University of Medical Sciences, Iran. For two years, 114 patients with CF (70 boys, 44 girls with the age range from two months to 15 years (average age: 31.8 months) who were referred to the Children's Medical Center Hospital, The Pediatrics Center of Excellence in Tehran, have been enrolled. The diagnosis of CF was made based on clinical manifestations, accompanied by an elevated sweat chloride concentration. Inclusion criteria were based on two consecutive positive sweat chloride tests. Diagnosis was made based on pilocarpine method with values > 60 mmol/l and chronic pulmonary disease and pancreatic insufficiency (9). Children evidence of liver disease as hepatitis B and C, α1 antitrypsin deficiency, and Wilson's disease were excluded. Since the research subjects were under 18, an informed consent was obtained from the parents of the patients. The study was approved by the hospital committee on ethics and human experimentation.

3.2. Biochemical Tests

A standard set of liver function tests including AST, ALT and GGT levels were performed, while abdominal US was done to assess liver architecture and a clinical examination. When two consecutive occasions, ALT and/or AST levels were ≥ 2 times the upper limit of normal values, biochemical liver disease is considered. For abnormal GGT, normal values by patient's age of the laboratory are considered. The interval between two examinations was about 2-6 weeks.

3.3. Abdominal US

Abdominal US and biochemical tests were performed on the same day. US was performed without sedation after a 4-hour fast in children aged 2-60 month and after an 8-hour fast in patients older than 60 months. US assessed the entire abdomen as well as a detailed examination of liver architecture. One of the following commercially available machines was used: (Quantum II (Siemens Medical Systems, Erlangen, Germany with a 3.5, 5.0, or 7.0-MHz transducer). The sonograms were obtained by one of pediatric radiologists and were later reviewed by another. In total, the whole US was obtained by two radiologists. In the case of inter observational variety; a third radiologist confirmed the reports. No radiologist was aware of the biochemical results at the time of examination or review.

3.4. Definitions of US Findings

US signs were interpreted as follows: Normal, hepatomegaly, increased echogenicity of liver, hepatomegaly accompanied by increased echogenicity of liver, increased echogenicity of liver and contracted gallbladder, sludge and calculus in the gallbladder, portal hypertension and intussusception. Liver abnormality was defined as hepatomegaly greater than 2 cm below the rib margin in the

right mid-clavicular line or an enlarged left lobe of liver according to US. The liver was called with increased echogenicity of liver if it was brighter than the cortex of the right kidney and if the walls of portal veins were difficult to discriminate from the adjacent liver parenchyma (10). Portal hypertension was as follows: enlargement of the portal vein, increased congestion index, and development of portosystemic collaterals (11).

3.5. Statistics

We summarized data using standard descriptive indices (mean, standard deviation, median, and range). The difference of the average age between groups was used by independent T test. Relationships between the prevalence of sonographically abnormal liver architecture and the prevalence of abnormal liver function test results were sought by using the $\chi 2$ test and accepting a P value less than 0.05 as significant. For analyzing our data, SPSS V-19 software was used.

4. Results

4.1. Demographic Data

Totally, 114 children patients have been recruited. Among them, 44 patients were female and 70 were male. The male predominance was not significant. The age of patients was in the range of 2 months to 180 months with the average age of 31.8 months. The standard deviation for the age was 49.2 months.

4.2. US Findings

Liver sonogram was abnormal in 42 patients (37.7 %), whereas 71 patients (62.3%) had normal liver architecture. The average age of patients with normal US was 30.5 in comparison to 33.9 in patients with abnormal US without any significant difference. Among the patients with abnormal US, the highest average age was for portal hypertension (117 months) which represents its late manifestation. Among the patients with abnormal sonography, seven patients (6.1%) had liver with hepatomegaly. Ten patients (8.8%) had increased echogenicity of liver, fourteen patients (12.3%) had both hepatomegaly and increased echogenicity of liver, three patients (2.6%) had contracted gallbladder and increased echogenicity of liver, four patients (3.5%) had sludge and calculus in gallbladder, four patients (3.5%) had portal hypertention and one patient (0.9%) had intussusception.

4.3. Biochemical Findings

The mean value of AST was 87.07 ± 105.7 U/L with the minimum and maximum of 16 U/L and 658 U/L respectively. The mean value of ALT was 48.31 ± 55.79 U/L with the minimum of 10 U/L and maximum of 338U/L. The mean value of GGT was 119.44 ± 134.82 U/L with the minimum of 7 U/L and maximum of 750 U/L. The AST level was elevated in 33 (28.9%) of the 114 patients. Moreover, elevated ALT and GGT levels were detected in 17 patients (14.9%) and 36 patients (31.6%), respectively. Twenty- two (51%) of 43 patients with abnormal sonography had at least one abnormal liver function test. Twenty – two (30.9%) of 71 patients with normal sonography had at least one abnormal liver function test. The difference between the two groups was statistically significant ($P < 0.05$). Therefore, children with abnormal livers in US had a much higher prevalence of biochemical abnormality. Besides, by categorization of the patients based on liver function test and US normality, forty-nine (42.9%) of 114 patients with CF had both normal US and liver function test; whereas sixty-five (57.1%) patients had at least one abnormality in US or Liver Function Tests (LFTs). The status of LFT results in different liver US abnormalities has been presented in the *Table 1*. The Pearson chi-square between Liver US finding and ALT, AST and GGT was 0.1, 0.2 and 0.01 respectively.

Table 1. Status of Liver Function Test in Different Liver US Findings [a]

Liver US Finding	GGT		AST		ALT	
	Normal	Increased	Normal	Increased	Normal	Increased
Normal, No. (%)	53 (74.6%)	18 (25.4%)	54 (76.1%)	17 (23.9%)	65 (91.5%)	6 (8.5%)
Hepatomegaly, No. (%)	6 (85.7%)	1 (14.3%)	5 (71.4%)	2 (28.6%)	5 (71.4%)	2 (28.6%)
IncreasedEchogenicity ofLiver, No. (%)	3 (30%)	7 (70%)	4 (40%)	6 (60%)	7 (70%)	3 (30%)
Hepatomegaly+IncreasedEchogenicity ofLiver, No. (%)	7 (50%)	7 (50%)	9 (64.3%)	5 (35.7%)	9 (64.3%)	5 (35.7%)
ContractedGallbladder, No. (%)	3 (100%)	0 (0.0%)	2 (66.7%)	1 (33.3%)	3 (100%)	0 (0.0%)
Sludge and Calculus in Gallbladder, No. (%)	4 (100%)	0 (0.0%)	4 (100%)	0 (0.0%)	4 (100%)	0 (0.0%)
PortalHypertention, No. (%)	1 (25%)	3 (75%)	2 (50%)	2 (50%)	3 (75%)	1 (25%)

[a] One patient had invagination in US finding which was not included in Table 1.

5. Discussion

This study was performed to assess liver abnormalities in a group of patients with CF. Among enrolled patients, 61.4% were male and 38.6% were female. Although no sex difference is expected, as a pattern of inheritance of disease, such male predominance was also previously reported (12, 13). CF is an autosomal recessive disorder, characterized by broad variability in clinical features due to genotype variation (14). European Epidemiologic Registry of CF (ERCF) has considered some functional mutation in CF which is related to the spectrum of clinical characteristics. A previous study investigated in the Iranian Children with CF indicated that the most common features of the disease were gastrointestinal disorders and respiratory manifestations (15). In this regard, genotype-phenotype correlation studies have shown higher incidence of liver disease in patients carrying mutations associated with a severe phenotype (16, 17). In addition, it has been reported that patients with CF liver disease are at increased risk for deterioration of nutritional status (18). Consequently, liver involvement in CF would be an important diagnostic and therapeutic challenge whose detection should be focused in the first decade of life. Although liver biopsy has been considered as the gold standard for assessing stage and grade of most chronic liver diseases, recently more noninvasive tests have been investigated (19). Potter et al. have described a clinical score including clinical factors and liver enzymes to predict the type of liver disease in CF with a sensitivity of 85% and a specificity of 82%, whereas its score did not evaluated US abnormality (20). It seems that by delineation of the liver architecture by US, the prevalence of liver disease in children with CF will be augmented. Our study focused on the early evolution of biochemical and ultrasound features of liver disease in a cross-sectional study of Iranian children younger than fifteen years old with CF. An initial assessment revealed that 37.7% of CF had liver ultrasound abnormalities in which 19.29% had coincided at least one biochemical abnormality. Previous cross-sectional studies showed a great abnormality in liver US and biochemical liver function tests in patients with CF (21, 22).

In this aspect, an epidemiologic cohort study has illustrated a prevalence of 41% of liver disease in CF patients which mainly occurs in the first decade of life (23). Besides, Ling et al. have reported a prevalence of 42% abnormality in biochemical and 35% in US findings of liver in 124 CF children of the UK at the initial assessment, in which by longitudinal follow up; abnormal biochemistry preceded or coincided with clinical or US abnormality in 74% developed new abnormalities (22). Since only end-stage liver disease as portal hypertension and cirrhosis would be with evident signs, Debray et al study recommended annual screening of liver by US and LFT for best practice guidance before any complication (24). In our study, 21 out of 114 patients (18.42%) had normal liver marker with abnormal US. This result in support of this finding, proposes early detection of liver disease by US which would be supportive in CF population regardless of liver marker tests. Therefore, routine use of US in annual assessment allows detection of a minority of CF patients with liver involvement but with normal biochemistry. However, our study suggested a higher prevalence of biochemical abnormality in patients with abnormal livers architecture in US, some previous researchers found no relation between liver US and function (25, 26). By considering the US abnormality more closely, the patients with increased echogenicity of liver in US have the most abnormality in LFT [N = 8 (80%) with at least one marker abnormality]. Besides, in patients with portal hypertension, the rate of abnormality in at least one marker was 75%. By examining the liver markers separately, GGT has the most abnormality among liver marker while 70% and 75% of cases had increased echogenicity of liver and portal hypertension in US, respectively. Therefore, among the liver markers, GGT has the most deviation in liver US abnormality. Previous studies mentioned an increase of liver disease in CF patients by aging (13, 27). In our study, approximate 43% of CF patients had no abnormality in LFT and US. The average age of mentioned group was 28.4 months vs. 36.34 months in group with at least one abnormality in US or LFT; however the difference in mean age was not significant. In this respect, a previous study on 450 CF patients unveiled that the liver disease had its onset during childhood in most cases (12). Furthermore, Lindblad et al study reported no more frequent liver disease at the end of a 15-year follow-up in a well-controlled population of patients with CF (28). Hence, it may be concluded that the patients who had no abnormality in liver either marker or US in infancy would be at lower risk of liver involvement later.

Limitation of our clinical study is inherent to US technique due to inter observer variability and low sensitivity in early stages. In addition, we did not consider the intermittent abnormality in LFT and liver US which has been mentioned before (22, 29). Future longitudinal studies could be recommended with a larger sample size to identify different liver US abnormalities associated with diverse liver marker tests. These data, although not conclusive due to the limited number of patients with abnormal liver architecture at US in each subtype, indicate LFTs in Iranian children with CF are related to US abnormality. The most specific US abnormalities related to abnormal function were signs suggestive of portal hypertension and the most abnormal marker was GGT. Hence, prediction of Ultrasound abnormalities by biochemical tests in our study provides support for their use in the early recognition of hepatobiliary involvement in CF.

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Authors' Contribution

All the authors contributed to the intellectual content of the study. Mehri Najafi, Nima Rezaei and Maryam Shoaran proposed the study. Maryam Shoaran and Farnaz Najmi Varzaneh collected the data and wrote the first draft. Nima Rezaei, Maryam Shoaran and Farnaz Najmi Varzaneh contributed in review of literature and data analysis. Mehrzad Mehdizadeh and Houman Alizadeh performed sonography. Maryam Shoaran and Farnaz Najmi Varzaneh submitted the paper.

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