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Early Diagnosis of Hepatobiliary System Lesions In Children With Cystic Fibrosis

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Abstract

Background

An increase in the life expectancy of cystic fibrosis patients contributes to the formation of severe hepatobiliary pathology, leading to the development of biliary cirrhosis with a fatal outcome. The purpose was to prospectively assess the predictive value of a combination of serum liver enzymes, ultrasound liver parameters, and transient elastography to diagnose clinically significant liver fibrosis. Materials and methods. 108 children aged 0-17 years with cystic fibrosis were examined. The fibrosis stage was determined using transient elastography on FibroScan®502 (Echosens, France). The activity of enzymes (alanine transaminase, aspartate transaminase, alkaline phosphatase, gamma-glutamyl transferase, lactate dehydrogenase-5), ultrasound parameters of the liver at different stages of liver fibrosis have been investigated. Results.

Liver fibrosis of varying severity was detected in 29.6% of patients with cystic fibrosis (liver elasticity ranged from 5.9 to 49.0 kPa). Liver cirrhosis was observed in 14.8% of children with cystic fibrosis. The dependence of an increase in the activity of alkaline phosphatase, gamma-glutamyl transferase, lactate dehydrogenase-5, and an enlargement of the left lobe of the liver, a reduction in the k ratio of the sizes of the right and left lobes of the liver on the degree of fibrosis F1-F4 ($p<0.05$) was found. Conclusions. The combined use of transient elastography FibroScan with increased activity of the alkaline phosphatase, gamma-glutamyl transferase, lactate dehydrogenase-5, and changing of ultrasound liver parameters could be used for early diagnosis of hepatobiliary lesions in cystic fibrosis. The age of a patient with cystic fibrosis over 6 years old, male gender, and the presence of ΔF508 deletion in the genotype have a high positive predictive value for liver fibrosis and cirrhosis.

Keywords: children; cystic fibrosis; hepatobiliary system; liver fibrosis; cirrhosis.

Introduction

Cystic fibrosis (CF) is the most common monogenic autosomal recessive metabolic disorder among people of European descent. It is characterized by a progressive course, disruption of vital bodily functions, early development of complications, premature disability in children, and a high mortality rate **[1]**. In Ukraine, approximately 300 CF patients are born annually, with a 5% frequency of heterozygous carriers of the CF gene in the population. Over the past decade, due to a better understanding of pathogenesis, improved diagnostics, and therapeutic-rehabilitative measures, the average lifespan of CF patients has significantly increased: from 5 years in 1970 to 24 years in Ukraine and over 45 years in Western European countries in 2016 **[1-3]**.

It has been established that the increased lifespan of CF patients contributes to the development of severe pathology of the hepatobiliary system, leading to the development of fatal biliary cirrhosis **[2-5]**. Literature reports indicate a high frequency of liver involvement in CF (ranging from 20% to 80%) with a characteristic asymptomatic course **[5,6]**. It is known that clinical manifestations of biliary disorders occurred in the stage of established cirrhosis, indicating a late diagnosis of the pathology of a biliary system **[4,7]**. There is a suggestion of a correlation between hepatomegaly and the severity of histological changes in the liver **[5,8]**. From this perspective, it is essential to determine the characteristics of hepatobiliary system involvement in CF and develop criteria for detecting pathological changes at the early stages of the disease. The need for such research is supported by the work of several authors **[8-13]**. However, the question of the patterns of biliary pathology development in CF remains debatable. There is a high frequency of liver involvement in males, and there are differing opinions regarding the connection between liver involvement and CF gene mutations **[5-8]**.

The presented data provide a basis for an in-depth study of the clinical features of hepatobiliary system involvement in CF and the search for adequate methods of early detection and determination of its severity.

Further prospects for improving treatment regimens are associated with developing strategies to prevent the progression of the pathological process in the liver. This, in turn, will enable an extension of the life expectancy of CF patients while enhancing their quality of life.

The purpose of the study was to prospectively assess the predictive value of a combination of serum liver enzymes, ultrasound liver parameters, and transient elastography for diagnosis of clinically significant liver fibrosis in CF patients.

Materials and Methods

From 2010 to 2020, 108 children aged 0 to 17 with CF were examined at the Cystic Fibrosis Center within the Odesa Regional Children's Clinical Hospital (Ukraine). The research was conducted by the principles of GCP (Good Clinical Practice) the Helsinki Declaration (1964, with amendments in 2013), and was approved by the local hospital's bioethics committee. Informed consent from the parents of the children (or their guardians) was obtained for participation in the study. Inclusion criteria for the study were: CF diagnosis; patient age between 0 and 17 years; absence of other chronic comorbidities; informed consent from parents and the child for participation in the research. Exclusion criteria from the study were severe congenital or acquired comorbidities and lack of support from parents and the child for participation in the study.

All patients underwent traditional clinical examination methods, and the results of general clinical laboratory and biochemical blood, urine, and stool tests were studied. Additionally, abdominal ultrasound diagnostics, esophagogastroduodenoscopy, and a pilocarpine iontophoresis sweat test were conducted. The activity of serum enzymatic markers of hepatobiliary pathology, including alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), and lactate dehydrogenase-5 (LDH-5), were assessed. The functional state of hepatocytes was evaluated using data from ultrasonic scanning of the biliary tract with the use of Doppler sensors, radioisotope hepatoscintigraphy with technetium-99m-labeled colloid, and transient elastography on the FibroScan®502 (Echosens, Paris, France). Elastography allowed for a quantitative assessment of liver stiffness in kilopascals (kPa) and determination of the fibrosis stage according to the METAVIR scale (liver stiffness ≤5.8 kPa corresponded to absence of fibrosis (F0), 5.9-7.0 kPa – F1 fibrosis, 7.1-9.4 kPa – F2 fibrosis, 9.5-12.5 kPa – F3 fibrosis, >12.5 kPa – F4 fibrosis, or liver cirrhosis) **[14,15]**. A liver biopsy was not performed due to its invasiveness and the high risk of complications.

Statistical data analysis was conducted using the software package Statistica 10 (StatSoft Inc., USA) and the online calculator SISA (Simple Interactive Statistical Analysis). To test statistical hypotheses regarding differences in relative frequencies in two independent samples, odds ratios and the achieved significance level (p) were calculated. Differences were considered non-random when the probability was $p<0.05$.

Results

From 2010 to 2020, 108 children aged 0-17 with CF were examined. The distribution of patients by gender showed a slight predominance of boys (53.7%), and the majority of children belonged to the preschool age group: 0-3 years (18.5%), 4-6 years (31.5%), 7-9 years (27.8%), and 10-17 years (22.2%). General clinical examination revealed hepatomegaly, with the liver enlarged by 1 to 5 cm below the costal margin in 98 patients (90.7%). Among them, 16 (14.8%) exhibited an acute poorly mobile edge. In 32 children (29.6%), the liver was firm in consistency and painless, while in 10 (9.3%), it had an uneven, nodular surface. Enlargement of the spleen from 1 to 7 cm was observed in 8 children (7.4%). On ultrasound examination, most patients (88.9%) exhibited heterogeneous acoustic structures of the liver. Clinical manifestations of hepatobiliary system involvement in children with CF were characterized by a mild and latent course, and the results of routine hematological and biochemical studies were not informative for specifying pathological changes in the liver parenchyma. Diagnosis of structural abnormalities was only possible through modern visualization methods. The distribution of CF children by the severity of liver fibrosis according to transient elastography (FibroScan, Echosens, France) was as follows: F0 (absence of fibrosis) - 76 patients (70.4%), F1 (minimal fibrosis) - 3 children (2.8%), F2 (moderate fibrosis) - 5 children (4.6%), F3 (severe fibrosis) - 8 children (7.4%), F4 (liver cirrhosis) - 16 patients (14.8%). The results showed the absence of liver fibrosis in 70.4% of CF patients, while 29.6% exhibited fibrotic changes of varying severity, with liver stiffness values ranging from 5.9 to 49.0 kPa. Among them, 16 children (14.8%) had minimal, moderate, or severe fibrosis, and 16 children (14.8%) had liver cirrhosis.

Further analysis of the patterns of hepatobiliary system involvement in children with CF was based on the study of medical and biological factors, including the age of the CF patient, the child's gender, and the presence of the ΔF508 mutation in homozygous or heterozygous state in compound with other modifications.

When categorizing CF patients by age, a trend of worsening hepatobiliary system pathology with increasing life expectancy was observed. Among children aged 0 to 3 years, hepatobiliary involvement (fibrosis F1-F4) was noted in 10% (95% confidence interval (CI) 3.14–23.14%), among children aged 4 to 6 years - in 29.41% (95% CI 13.74–44.25%), among children aged 7 to 9 years - in 33.33% (95% CI 16.17–49.82%), and among children aged 10 to 17 years - in 41.67% (95% CI 22.25–61.74%). These findings indicate a correlation between the severity of biliary pathology in CF and the disease duration.

A significant predominance of males among CF patients with hepatobiliary system involvement was established: fibrosis (F1-F4) was detected in 41.38% of boys (95% CI 29.29–54.70%) and 15.38%

of girls (95% CI 5.10–24.89%). The odds ratio for the developing biliary pathology in male CF patients compared to females was 3.7 (95% CI 1.47–9.29), and the relative risk of liver involvement was 2.59 (95% CI 1.24–5.85). Male gender in CF patients should be considered an adverse prognostic factor for the development of pathological changes in the liver.

The analysis of CF patient genotypes revealed that among homozygotes for the ΔF508 deletion, the frequency of hepatobiliary system involvement was 43.33% (95% CI 30.47–55.52%), among heterozygotes with the ΔF508 deletion in compounds with other mutations - 11.76% (95% CI 1.07–22.92%), and among patients with mutations other than ΔF508 - 14.28% (95% CI 4.17–32.17%). The odds ratio for developing biliary pathology in CF patients with the ΔF508 deletion in their genotype was 2.81 (95% CI 0.59–13.36), and the relative risk was 2.23 (95% CI 0.67–13.25). The ΔF508 mutation of the CF gene should be considered when assessing the likelihood of hepatobiliary system involvement.

Table 1: Serum blood enzyme activity in children with CF (mean±SD)

Fibrosis stage	ALT, IU/L	AST, IU/L	ALP, IU/L	GGT, IU/L	LDH-5, %
F0 n=76	23,5±1,3	18,7±2,1	320,3±9,3	28,6±3,1	7,6±0,4
F1-F3 n=16	28,7±4,7	29,5±2,4	455,8±39,3*[1]	54,3±8,1*[1]	23,0±1,8*[1]
F4 n=16	72,4±14,8*[2]	69,6±2,8*[2]	628,1±47,5*[2]	128,3±21,2*[2]	32,3±4,3*[2]

Notes: *[1] – statistical significance of differences between fibrosis stages F0 and F1-F3 ($p < 0.05$),

*[2] – statistically significant differences between fibrosis stages F1-F3 and F4 ($p<0.05$).

The ALT and AST enzyme activity levels remained within normal ranges in children with CF and fibrosis stages F0-F3 but significantly increased in those with cirrhosis (F4) ($p<0.05$). Therefore, standard biochemical tests could not detect liver pathology in CF children at early stages.

Increased levels of ALP, GGT, and LDH-5 were found in CF patients, which significantly differed from the values in healthy children ($p<0.001$). It was noted that the elevation of ALP, GGT, and LDH-5 enzyme concentrations depended on the degree of fibrosis, with the highest activity observed in cirrhosis ($p<0.05$).

The obtained results allowed for the establishment of a correlation between the elevation of ALP, GGT, and LDH-5 activity and the severity of hepatobiliary pathology in CF children. ALP activity levels of 320.3±9.3 (95% CI 295.2–345.4) IU/L, GGT activity of 28.6±3.1 (95% CI 22.2–35.0) IU/L, and LDH-5 activity of 7.6±0.4 (95% CI 5.5–9.7) % may indicate the absence of liver fibrosis (F0) in CF patients. Elevated enzyme activity of ALP 455.8±39.3 (95% CI 384.4–527.2) IU/L, GGT 54.3±8.1 (95% CI 36.8–71.8) IU/L, and LDH-5 23.0±1.8 (95% CI 17.8–28.2) % suggests the possibility of fibrosis (F1-F3) and indicates the need for transient elastography of the liver. Increased ALP activity of 628.1±47.5 (95% CI 541.4–714.8) IU/L, GGT 128.3±21.2 (95% CI 79.1–177.5) IU/L, and LDH-5 32.3±4.3 (95% CI 22.5–42.1) % with a high probability indicates the presence of liver cirrhosis (F4) and is an indication for performing elastography to determine the degree of fibrosis according to the METAVIR scale.

The study of parameters related to the liver's transverse dimensions and the diameters of hepatic veins using ultrasound scanning allowed for the determination that the progression of pathological processes in the hepatobiliary system in CF children was associated with an enlargement of the left lobe of the liver. Moreover, a significant increase in the size of the left lobe corresponded to a higher degree of fibrosis ($p<0.05$). A reverse correlation was observed between the k coefficient (the ratio of the sizes of the right and left lobes of the liver) and the degree of fibrosis, with a greater degree of fibrosis corresponding to a smaller value of this parameter ($p<0.05$). The maximum enlargement of the left lobe of the liver, the smallest value of the k coefficient, and the dilation of the diameters of the portal and splenic veins were observed in patients with liver cirrhosis: **Table 2**.

The determination of the prognostic value of the studied medical-biological factors regarding the development of liver fibrosis in CF revealed high sensitivity, moderate specificity, and predictive value for the following parameters: age of the CF patient over 6 years (0.63, 0.55, and 0.37, respectively), male gender of the child (0.75, 0.55, and 0.41, respectively), and the presence of the ΔF508 deletion in the genotype (0.88, 0.37, and 0.37, respectively). It is recommended to use the proposed criteria for identifying high-risk groups for developing hepatobiliary pathology in CF children. The application of modern enzymatic analysis technologies and instrumental visualization methods with quantitative interpretation of liver parenchyma elasticity has become the basis for creating a differentiated system for assessing the state of the hepatobiliary system in CF children.

The study of ALT, AST, ALP, GGT, and LDH-5 enzyme activity was conducted depending on the degree of liver fibrosis: **Table 1**.

Table 2: Increase in liver cross-sectional dimensions and diameters of hepatic veins in children with CF (mean±SD)

Fibrosis stage	Enlargement of the right lobe of the liver, mm	Enlargement of the left lobe of the liver, mm	Coefficient k of the liver lobes ratio	Portal vein dilation, mm	Splenic vein dilation, mm
F0 n=76	0,40±0,03	0,40±0,02	1,60±0,10	0,0±0,0	0,0±0,0
F1-F3 n=16	4,80±1,10*[1]	19,4±1,1*[1]	1,30±0,08*[1]	0,0±0,0	0,0±0,0
F4 n=16	0,20±0,02	31,5±1,4*[2]	1,00±0,30*[2]	2,5±0,5*[2]	1,9±0,3*[2]

Notes: *[1] – significance of differences between fibrosis stages F0 and F1-F3 ($p<0.05$),

*[2] – significance of differences between fibrosis stages F1-F3 and F4 ($p<0.05$).

The obtained data support the rationale for using ultrasound diagnostics to detect pathological changes in the hepatobiliary system in CF at early stages of development. In a child with CF, an increase in the left liver lobe of 19.4±1.1 (95% CI 14.2–24.6) mm compared to age norms and a decrease in the k coefficient of the right-to-left liver lobes to 1.30±0.08 (95% CI 0.9–1.7) may indicate the development of fibrosis F1-F3 and is an indication for liver elastography. An increase in the left liver lobe by 31.5±1.4 (95% CI 24.9–38.1) mm beyond age norms, a k coefficient value of 1.0±0.3 (95% CI 0.5–1.5), an expansion of the portal vein diameter by 2.5±0.5 (95% CI 1.4–3.6) mm, and splenic vein diameter by 1.9±0.3 (95% CI 1.0–2.8) mm suggest cirrhosis of the liver (F4) and indicate the need for elastography to refine the degree of fibrosis according to the METAVIR scale.

The study of the prognostic value of the proposed criteria for early diagnosis of liver fibrosis and cirrhosis in CF patients revealed that the ultrasound parameters of the liver had the highest predictive value: enlargement of the left liver lobe (0.75), the k coefficient of the right-to-left liver lobes (0.80), and the expansion of the portal vein and splenic vein diameters (0.88). It also confirmed the high sensitivity (0.94) and moderate prognostic value of the serum levels of LF, GGT, and LDH-5 enzymes.

Discussion

The use of advanced medical technologies, aggressive therapeutic regimens, and transplantation programs in CF patients has become an objective factor contributing to the increase in the frequency of late complications of the disease. Liver involvement with the development of fibrosis and cirrhosis is the second leading cause of death after lung complications **[1-4]**.

In-depth research into the features of liver involvement was conducted in 108 children with cystic fibrosis (CF). Clinical manifestations of biliary pathology differed with a mild and latent course, and the results of routine biochemical tests were not informative for detecting pathological changes in liver parenchyma. Diagnosis of structural abnormalities required modern imaging methods to determine fibrosis severity using the METAVIR scale.

The conducted transient elastography of the liver (FibroScan, Echosens, France) showed the absence of fibrosis in 70.4% of CF patients and the presence of fibrotic changes of varying severity in 29.6% of CF children (with a range of liver elasticity median values from 5.9 to 49.0 kPa), with half of them (14.8%) having cirrhosis of the liver. The results indicate a high frequency (in every third patient) of biliary pathology in CF, which can lead to a fatal outcome consistent with previous research findings **[3-6]**.

The analysis of the patterns of hepatobiliary system involvement in children with CF allowed for the identification and justification of the use of specific medical and biological factors (age, gender, the presence of the ΔF508 mutation) as prognostic criteria for the development of liver fibrosis and cirrhosis.

The trend of progressing pathological changes in the liver with increasing patient lifespan has been established: liver involvement was observed in 10% of children aged 0-3 years, 29.41% of children aged 4-6 years, 33.33% of children aged 7-9 years, and 41.67% of children aged 10-17 years. These findings confirm the association between the severity of biliary pathology in CF and the duration of the disease. These results align with existing literature on the increasing frequency and severity of hepatobiliary system involvement in CF with prolonged patient survival **[5-8]**.

The study revealed a significant predominance of males among CF patients with hepatobiliary system involvement: fibrosis (F1-F4) was observed in 41.38% of boys and 15.38% of girls. Therefore, liver involvement in CF occurs much more frequently in boys than in girls. The odds ratio of developing biliary pathology in male CF patients compared to females was 3.7 (95% CI 1.47–9.29), and the relative risk of liver involvement was 2.59 (95% CI 1.24–5.85). Male gender in CF patients is considered an unfavorable prognostic factor for developing pathological liver changes. These results are consistent with findings from some authors, indicating a high frequency of hepatobiliary system involvement in males and the predominance of cirrhosis formation in boys **[3,5]**.

The study confirmed a high frequency of hepatobiliary system involvement among homozygotes for the ΔF508 deletion (43.33%) compared to heterozygotes with the ΔF508 deletion in compounds

with other mutations (11.76%) and among patients with mutations other than ΔF508. The odds ratio for developing biliary pathology in CF patients with the ΔF508 deletion was 2.81 (95% CI 0.59–13.36), and the relative risk was 2.23 (95% CI 0.67–13.25). It is known that among the studied mutations of the CF gene, there is no specific mutation that directly causes liver involvement **[5,6]**. However, when a CF patient has the ΔF508 deletion in their genotype, it should be considered a high probability of hepatobiliary system involvement.

The progression of pathological changes in the hepatobiliary system in CF was accompanied by an increase in the activity of serum enzymes, including ALP, GGT, and LDH-5. It was found that there is a direct relationship between the increase in their concentration and the degree of fibrosis from F1 to F4 ($p<0.05$). The detection of elevated activity of ALP, GGT, and LDH-5 in CF patients may indicate the development of biliary pathology and is an indication for performing transient elastography to determine the degree of fibrosis. These findings are consistent with the results of previous scientific studies **[11-13,16]**.

The progression of biliary pathology in CF patients was associated with an increase in the left lobe of the liver and a decrease in the coefficient k (the ratio of the sizes of the right and left lobes of the liver). A relationship was found between these deviations and the degree of fibrosis from F1 to F4 ($p<0.05$), which is in line with reports from some researchers **[16,17]**. Detecting an enlargement of the left lobe of the liver and a reduction in the coefficient k in CF patients allows for suspicion of fibrosis development. It is an indication for performing transient elastography to determine the degree of fibrosis according to the METAVIR scale.

Conclusions

The combined use of transient elastography FibroScan with increased activity of the ALP, GGT, and LDH-5 enzymes and changing of ultrasound liver parameters (enlargement of the left lobe of the liver, a reduction in the k ratio of the sizes of the right and left lobes of the liver, dilatation of the portal and splenic veins) could be used for early diagnosis of hepatobiliary lesions in cystic fibrosis. The age of a patient with cystic fibrosis over 6 years old, male gender, and the presence of ΔF508 deletion in the genotype have a high positive predictive value for liver fibrosis and cirrhosis.

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Authors` Contribution:

All authors were involved in the conception and design of the study; acquisition of data; formal analysis and interpretation of data; critical revision of the manuscript for intellectual content and final approval of the submitted and published version; in addition the authors Y. Tsyunchyk and I. Shevchenko have performed the drafting of the manuscript and statistical analysis.

References

1. Dodge JA, Lewis PA, Stanton M, Wilsher J (2007) Cystic Fibrosis mortality and survival in the United Kingdom, 1947 to 2003. Eur. Respir. J. 29(3): 522-526.
2. Colombo C, Zazzeron L, Lanfranchi C, Daccò V (2021) Liver Disease in Cystic Fibrosis. In book: Diseases of the Liver and Biliary Tree. pp.93-113.
3. Thavamani A, Sankararaman S, Sferra T (2022) Association between cystic fibrosis–related liver disease, mortality, and disease burden in children. J Cyst Fibros. 21: 113-114.
4. Stonebraker JR, Ooi CY, Pace RG, Corvol H, Knowles MR, et al. (2016) Features of severe liver disease with portal hypertension in patients with cystic fibrosis. Clin Gastroenterol Hepatol. 14(8): 1207-1215.e3.
5. Colombo C (2007) Liver disease in cystic fibrosis. Curr Opin Pulm Med. 13(6): 529-536.
6. Debray D, Kelly D, Houwen R, Strandvik B, Colombo C (2011) Best practice guidance for the diagnosis and management of cystic fibrosis-associated liver disease. J. Cyst. Fibros. 10 (Suppl 2): S29-36.
7. Herrmann U, Dockter G, Lammert F (2010) Cystic fibrosis-associated liver disease. Best Pract Res Clin Gastroenterol. 24(5): 585-592.
8. Rowland M, Bourke B (2011) Liver disease in cystic fibrosis. Curr Opin Pulm Med. 17(6): 461-466.
9. Pereira TN, Lewindon PJ, Smith JL, Murphy TL, Lincoln DJ, et al. (2004) Serum markers of hepatic fibrogenesis in cystic fibrosis liver disease. J Hepatol. 41(4): 576-583.
10. Wunsch E, Krawczyk M, Milkiewicz M, Trottier J, Barbier O, et al. (2016) Serum autotaxin is a marker of the severity of liver injury and overall survival in patients with cholestatic liver diseases. Sci Rep. 6: 30847.
11. Leung DH, Khan M, Minard CG, Guffey D, Ramm LE, et al. (2015) Aspartate aminotransferase to platelet ratio and fibrosis-4 as biomarkers in biopsy-validated pediatric cystic fibrosis liver disease. Hepatology. 62(5): 1576-1583.
12. de Lédinghen V, Le Bail B, Rebouissoux L, Fournier C, Foucher J, et al. (2007) Liver stiffness measurement in children using FibroScan: feasibility study and comparison with Fibrotest, aspartate transaminase to platelets ratio index, and liver biopsy. J Pediatr Gastroenterol Nutr. 45(4): 443-450.
13. Cook NL, Pereira TN, Lewindon PJ, Shepherd RW, Ramm GA (2015) Circulating MicroRNAs as Noninvasive Diagnostic Biomarkers of Liver Disease in Children With Cystic Fibrosis. J Pediatr Gastroenterol Nutr. 60(2): 247-254.

14. Bedossa P, Poynard T (1996) An algorithm for the grading of activity in chronic hepatitis C. The METAVIR cooperative study group. Hepatology. 24(2): 289-293.
15. Sandrin L, Fourquet B, Hasquenoph JM, Yon S, Fournier C, et al. (2003) Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. Ultrasound Med Biol. 29(12): 1705-1713.
16. Leung DH (2017) Leung Hepatic fibrosis scores and serum biomarkers in pediatric hepatology. Clin Liver Dis (Hoboken). 9(5): 125–130.
17. Friedrich-Rust M, Schlueter N, Smaczny C, Eickmeier O, Rosewich M, et al. (2013) Non-invasive measurement of liver and pancreas fibrosis in patients with cystic fibrosis. J Cyst Fibros. 12(5): 431-439.