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The contribution of imaging to non-invasive fibrosis biomarkers in the diagnosis and staging of chronic liver disease

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ABSTRACT

Aims: Gold standard technique for determining the stage of fibrosis in cirrhosis is a biopsy. Non-invasive tests are used when a biopsy is contraindicated. However, their specificity and sensitivity still fall short of expectations. Aim of the study is to develop a model capable of determining fibrosis using serum biomarkers and liver ultrasonography.

Methods: A retrospective study was designed including patients with chronic hepatitis B and C underwenting liver biopsies between the time frame of 2015 to 2020 years at Trakya University School of Medicine. Epidemilogical data, ultrasonography and pathology reports were noted. Blood values were recorded and used to calculate AST / Platelet Ratio Index (APRI), Fibrosis-4 Index (FIB-4), Gothenburg University Cirrhosis Index (GUCI) noninvasive fibrosis indices. The fibrosis stages of the patients were assessed accoridng to pathology reports into three categories: advanced (F5-F6), moderate (F3-F4), and lower Ishak scores.

Results: A total of 259 patients were included in the study. The median age of the patients was 54 (19-90), and 40.9% (106) were female. The median values of APRI, GUCI and FIB-4 scores were respectively: 0.6 (0-21.8), 0.6 (0-26.2) and 1.6 (0.2-8.5). The effects of ultrasonography findings were examined to improve the diagnostic performance of APRI, GUCI and FIB-4 indices. Accompanied by statistical analysis, it was observed that the FIB-4 index and the presence of hepatosteatosis in the liver had a significant effect on the detection of F≥3 (respectively; p<0.001, p=0.033). A new model named FIB4u (ultrasonography) was developed. The AUC values of indices for differentiation of intermediate and advanced stages of fibrosis (≥3) were respectively:FIB4u 0.760; FIB-4 0.753; GUCI 0.676; APRI 0.667 (p<0.001). The FIB4u index demonstrated considerably better performance compared to both APRI and GUCI.

Conclusion: The FIB4u index, developed by combining ultrasonography and laboratory data, can be used as a new index for fibrosis assessment in the absence of advanced elastography techniques. It needs to be validated in larger patient cohorts to be used safely in the long term.

Keywords: Cirrhosis, FIB4, non-invasive fibrosis indices, ultrasonography

INTRODUCTION

Worldwide, approximately two million people die each year due to liver diseases. One million of these deaths are due to complications of cirrhosis. Cirrhosis ranks 11th cause of death. Together, cirrhosis and HCC account for 3.5% of all fatalities worldwide. The mortality rate has increased by 0.5% since the year 2000.[1] It has been observed that as fibrosis progresses, cirrhosis development and viral, non-viral liver disease complications increase.[2,3] Therefore, it is important to be able to determine the stage of fibrosis. There are invasive (liver biopsy) and non-invasive (serum markers and imaging) methods for detecting fibrosis. Although liver biopsy is the best method, due to its interventional nature it can cause pain, morbidity and mortality. In addition, the distribution of fibrosis is heterogeneous, and tissue biopsies represent only 1:500000 of the entire organ. The stage of fibrosis can be interpreted variably by pathologists.[4]

There are two major classifications for non-invasive markers. Biological methods consisting of serum biomarkers and imaging techniques measuring liver rigidity. Many indices have been developed by using various combinations of markers and adding clinical parameters such as age, gender, and body mass index to the formulations. Some of these formulas are as follows: AST / Platelet Ratio Index (APRI), Fibrosis-4 Index (FIB-4), GUCI Gothenburg University Cirrhosis Index (GUCI), Hui score, Zeng score, ALT ratio. Ultrasonography

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(US) and magnetic resonance-based elastography are the methods that examine the liver parenchyma structure using a physical approach. The main principle is that the tissue stiffness increases as fibrosis increases.[5] The advantages of biomarkers are easy applicability, safe interlaboratory reproducibility, and widespread availability, especially of non-patent ones.[6,7] However, these markers have disadvantages such as not only reflecting liver specific fibrosis and their values can be affected in different physiological conditions and diseases. Similarly, elastography methods have disadvantages such as availability of special equipment, application problems (obesity, ascites, experience of the performer), failure to reflect intermediate fibrosis values, and false positive results (due to acute hepatitis, extrahepatic cholestasis, liver congestion, post meal).[5] Therefore, there is no ideal marker to predict fibrosis.

The aim of our research is to develop a model that can determine fibrosis with serum biomarkers and liver ultrasound features in patients with chronic liver disease and to predict the prognosis of liver disease without any intervention.

METHODS

Patients and Sample Collection

Our retrospective study included 259 patients over the age of 18 as participants, who underwent liver parenchymal biopsy and were monitored in the Gastroenterology department of Trakya University Medical Faculty Hospital between 2015 and 2020 with HBV and HCV diagnoses. The study was approved by the Trakya University Faculty of Medicine Scientific Researches Ethics Committee (Date: 10.08.2020, Decision No: 12/10). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Age, gender, chronic liver disease etiologies, blood sample results (hemoglobin, white blood cells, neutrophil, lymphocyte, platelet, total bilirubin, direct bilirubin, sodium, potassium, urea, creatinine, total protein, albumin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma glutamyl transpeptidase, prothrombin time, international normalized ratio, activated partial thromboplastin clotting time levels) were obtained from medical records of patients. Liver and spleen US was performed using Toshiba Aplio 500 and Esaote MyLab 70 model US device. Fibrosis scores from liver parenchymal biopsy reports were obtained from Pathology Department. ISHAK fibrosis scoring system was used. With laboratory results, APRI ((AST/AST upper limit) x (100/PLT)) score, GUCI score ((AST/AST upper limit) x INR x 100/PLT)) and FIB-4 ((Age x AST/(PLT x$\sqrt{}$ALT)) scores were calculated. Liver size, spleen size, liver heterogeneicty, microlobulation and hepatosteatosis findings were recorded from US reports.

Statistical Analysis

The normality condition for continuous variables was checked with the Shapiro-Wilk test. The Kruskal-Wallis test (Post-Hoc: Dunn-Bonferroni test) was performed when the data of the three groups did not exhibit normal distribution, and the one-way analysis of variance was performed otherwise. The relationship between two categorical variables was examined with Pearson's Chi-square test and Fisher's Exact test. The development work on the indices was carried out by logistic regression analysis. Receiver Operating Characteristic (ROC) curve analysis was performed and Area Under Curve (AUC) values were compared with DeLong test. Sensitivity, specifity, positive cut-off and negative cut-off values were calculated. Data were expressed as mean, standard deviation, median, minimum and maximum values. Statistical software SPSS version 23 (SPSS Inc., Armonk, NY) was used for all analyzes. R (Version 4.1.0) program ("pROC",Version 1.17.0.1) package was used for ROC analysis. The significance level was determined as $p<0.05$.

RESULTS

The median age of the patients was 54 (19-90). 40.9% (n=106) were female. Of the viral hepatitis etiology in the patients, 226 (87.3%) were HBV and 33 (12.7%) were HCV.

In the ultrasonography examination, spleen enlargement was detected in 6.2% (n=16), liver heterogenecity in 22.8% (n=59), liver microlobulation in 5% (n=13), liver hepatosteatosis in 12.7% (n=33) of patients (**Table 1**).

Table 1. Ultrasonography findings of the patients

	n	%
Spleen size		
Normal	243	93.8
Increased	16	6.2
Liver heterogeneity		
No	200	77.2
Yes	59	22.8
Liver microlobulation		
No	246	95.0
Yes	13	5.0
Liver hepatosteatosis		
No	226	87.3
Yes	33	12.7
Liver enlargement		
No	217	83.8
Yes	42	16.2

The median values of the patients' APRI, GUCI and FIB-4 scores were respectively; 0.6 (0-21.8), 0.6 (0-26.2) and 1.6 (0.2-8.5).

The distribution of the patients numbers (n) according to histopathological fibrosis stages (F) was as follows: F0, n=13; F1, n=42; F2, n=86; F3, n=60; F4, n=29; F5,n=28; F6, n=1.

According to the stages of fibrosis, patients were divided into three groups: advanced (F5-F6), intermediate (F3-F4), and lesser levels (F0-F1-F2). And analyses were conducted based on these groupings.

When the characteristics of the patients were examined in terms of the Ishak Fibrosis Score, a significant relationship was found with age. Those with fibrosis stage F5-F6 were significantly older than those with F0-F1-F2 and F3-F4 scores. Those with fibrosis stage F3-F4 were significantly older than those with F0-F1-F2 (KW: $\chi2=25.083$, $p<0.001$, Post-Hoc: respectively, $p=0.006$, $p<0.001$, $p=0.043$) (**Table 2**).

Table 2. Ishak fibrosis scores according to the characteristics of the patients

	Total (n=259)	Ishak Fibrosis Scores			Test p
		F0-F1-F2 (n=141)	F3-F4 (n=89)	F5-F6 (n=29)	
Age					
Mean±sd	52.4±13.6	48.8±14.3	55±10.9	61.8±11.3	<0.001*
Gender					
Female	106 (40.9)	57 (53.8)	37 (34.9)	12 (11.3)	0.984**
Male	153 (59.1)	84 (54.9)	52 (34)	17 (11.1)	

Sd:standard deviation, Med (Min-Max): Median (Minimum - Maximum), Kruskal Wallis test*, Pearson's chi-square test**

When laboratory values and fibrosis stages were compared, as expected, white blood cells, neutrophil and thrombocyte count, albumin values were significantly decreased in advanced fibrosis stages. Additionally in patients with a high stage of fibrosis, total bilirubin, direct bilirubin, urea, ast, alp, ggt, prothrombin time, and INR values were elevated (Table 3).

Table 3. Ishak fibrosis scores according to the laboratory values of the patients

	Ishak Fibrosis Scores			Test p*
	F0-F1-F2 (n=141) (mean±sd)	F3-F4 (n=89) (mean±sd)	F5-F6 (n=29) (mean±sd)	
Hemoglobin level (gr/dl)	14.4±1.6	14.3±1.5	13.9±1.6	0.319
White blood cells count	6.9±2	6.7±1.9	6±1.9	0.036
Neutrophil count	4±1.4	3.7±1.4	3.3±1.1	0.020
Lymphocyte	2.2±0.7	2.1±0.7	2±0.8	0.195
Platelet count (x10^3)	229.6±60.7	188.5±54.2	162±43.6	<0.001
Total Bilirubin	0.8±0.5	1±0.7	1±0.4	0.031
Direkt Bilirubin	0.3±0.3	0.3±0.5	0.4±0.3	0.001
Sodium	139.3±2.7	139±4.4	139.2±2.3	0.993
Pootassium	4.5±0.4	4.4±0.4	4.4±0.3	0.277
Urea	28.6±9.1	31.2±17.8	32.8±9.3	0.031
Creatinin	0.8±0.3	0.8±0.5	0.8±0.2	0.583
Total Protein	7.4±0.6	7.3±0.9	7.3±0.7	0.609
Albumin	4.2±0.4	4.1±0.4	3.9±0.5	<0.001
ALT	131.1±240.3	108.7±152.3	162.8±167.6	0.061
AST	84.1±153.7	75.8±88.4	109.9±91.2	<0.001
ALP	86.2±37.9	97.8±43.8	101.5±33.5	0.009
GGT	47.8±61.4	84.2±240.1	125±132.1	<0.001
Protrombin time	13.7±1.3	14.2±0.9	14.5±1.1	<0.001
INR	1±0.1	1.1±0.1	1.1±0.1	<0.001
APTT	28.4±2.7	28.9±2.6	29.2±2.4	0.333

Sd:standard deviation, Med (Min-Max): Median (Minimum - Maximum), ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase, ALP: Alkaline Phosphatase, GGT: Gamma glutamyl transpeptidase, INR: International Normalized Ratio, APTT: Activated Partial Thromboplastin Time. *Kruskal Wallis test, One-way analysis of variance

Liver ultrasonography findings and fibrosis stage were compared. Liver heterogeneity and microlobulation features were more observed in advanced fibrosis stages (p=0.043; p=0.004) (Table 4).

Table 4. Ishak fibrosis scores according to the ultrasonographic findings of the patients

	Total (n=259)	Ishak Fibrosis scoring			Test p*
		F0-F1-F2 (n=141)	F3-F4 (n=89)	F5-F6 (n=29)	
Spleen size					0.528
Normal	16 (6.2)	7 (43.8)	6 (37.5)	3 (18.8)	
Enlarged	243 (93.8)	134 (55.1)	83 (34.2)	26 (10.7)	
Liver heterogeneity					0.043
No	200 (77.2)	116 (58)	66 (33)	18 (9)	
Yes	59 (22.8)	25 (42.4)	23 (39)	11 (18.6)	
Liver microlobulation					0.004
No	246 (95)	139 (56.5)	82 (33.3)	25 (10.2)	
Yes	13 (5)	2 (15.4)	7 (53.8)	4 (30.8)	
Liver hepatosteatosis					0.303
No	226 (87.3)	127 (56.2)	74 (32.7)	25 (11.1)	
Yes	33 (12.7)	14 (42.4)	15 (45.5)	4 (12.1)	
Liver enlargement					0.660
No	217 (83.8)	117 (53.9)	74 (34.1)	26 (12)	
Yes	42 (16.2)	24 (57.1)	15 (35.7)	3 (7.1)	

Pearson's chi-square test, Fisher exact test*

When APRI, GUCI, and FIB-4 index values were examined, a significant difference was found between all fibrosis stage groups (Respectively; $\chi2$=31,346, $\chi2$=33,799, $\chi2$=59,460, $\chi2$=40.936, p<0.001) (Table 5).

Table 5. Ishak fibrosis scores according to fibrosis indices values

	Ishak fibrosis scoring			Test P*
	F0-F1-F2 (n=141) (mean±sd)	F3-F4 (n=89) (mean±sd)	F5-F6 (n=29) (mean±sd)	
APRI	1.2±2.5	1.3±1.5	2±1.4	<0.001
GUCI	1.2±2.8	1.4±1.7	2.2±1.5	<0.001
FIB-4	1.6±1.4	2.3±1.3	3.8±1.9	<0.001

Sd:standard deviation, Med (Min-Max): Median (Minimum - Maximum), APRI: AST / Platelet Ratio Index; GUCI: Gothenburg University Cirrhosis Index, FIB-4: Fibrosis 4 Index. *Kruskal Wallis test

Model Study with Ultrasonography Findings in Fibrosis Indices

The effects of ultrasonography findings were examined with logistic regresion analysis to improve the diagnostic performance of APRI, GUCI and FIB-4 indices for diagnosis of Ishak Fibrosis score ≥3 (F≥3).

As a result it was seen that APRI and GUCI indices were not significant in detecting F≥3 (respectively; p=0.348, p=0.321). Therefore, model development was not carried out.

In the analysis, it was observed that the FIB-4 index and the presence of hepatosteatosis in the liver had a significant effect in the detection of Fibrosis score ≥3 (Respectively; p<0.001, p=0.033) (Table 6).

Table 6. Logistic regression models for Ishak Fibrosis ≥3 with indices and ultrasonography findings

	Ishak Fibrosis score ≥3			p
	ß coefficient	SE	Odds	
APRI	0.061	0.065	1.063	0.348
Liver heterogeneity	-0.613	0.312	0.542	0.049
Liver microlobulation	1.964	0.787	7.128	0.013
Liver hepatosteatosis	-0.758	0.384	0.468	0.048
Constant	2.163	0.975	8.701	0.026
GUCI	0.060	0.060	1.061	0.321
Liver heterogeneity	0.603	0.312	1.828	0.053
Liver microlobulation	1.961	0.787	7.105	0.013
Liver hepatosteatosis	0.830	0.391	2.292	0.034
Constant	-0.580	0.179	0.560	0.001
FIB-4	0.534	0.109	1.706	<0.001
Liver microlobulation	1.535	0.810	4.639	0.058
Liver hepatosteatosis	0.897	0.396	2.452	0.024
Constant	-1.456	0.263	0.233	<0.001

SE: Standard error, APRI: AST / Platelet Ratio Index, GUCI: Gothenburg University Cirrhosis Index, FIB-4: Fibrosis 4 Index.

Although the presence of microlobulation in the liver does not have a statistically significant effect on the model, it is included in the final model due to its positive contribution to the model fit (p=0.058). The model named "FIB4u" is significant and the model related -2 Log Likelihood value of the model: 313.245, Cox & Snell R Square value: 0.155, Nagelkerke R Square value: 0.208, and goodness-of-fit (Hosmer and Lemeshow test) $\chi2$=14,237 and p=0.076 was found (p<0.001). The formula of the developed model is given below.

Formula for the detection of fibrosis ≥3:

$$FIB4u = \frac{1}{1 + e^{-(-1,456 + 0,534xFIB - 4 + 1,535xmicrolobulation\ \ in\ liver + 0,897xhepatosteatosis\ in\ liver)}}$$

The effects of ultrasound findings were examined in order to improve the diagnostic performance of APRI, GUCI and FIB-4 indices also for Ishak Fibrosis score ≥5 (F≥5). Analysis revealed that APRI and GUCI indices were not significant in detecting Fibrosis score ≥5 (Respectively; p=0.082, p=0.087). Therefore, model development was not carried out. FIB-4 index had a significant effect on the detection of Fibrosis score ≥5 (p<0.001), but the microlobulation status in the liver did not have a significant effect on the model (p=0.191). Therefore, a new model could not be developed to improve the performance of the FIB-4 index for fibrosis score ≥5 (Table 7).

Table 7. Logistic regression models for Ishak Fibrosis ≥ 5 with indices and ultrasonography findings

	Ishak Fibrosis score ≥5			p
	ß coefficient	SE	Odds	
APRI	0.120	0.069	1.128	0.082
Liver microlobulation	1.411	0.639	4.100	0.027
Sabit	-2.365	0.245	0.094	<0.001
GUCI	0.105	0.062	1.111	0.087
Liver microlobulation	1.395	0.639	4.036	0.029
Sabit	-2.349	0.242	0.095	<0.001
FIB-4	0.561	0.112	1.753	<0.001
Liver microlobulation	0.880	0.673	2.410	0.191
Sabit	-3.647	0.416	0.026	<0.001

SE: Standart error, APRI: AST / Platelet Ratio Index, GUCI: Göteborg University Cirrhosis Index, FIB-4: Fibrosis 4 Index.

Roc Curve Analysis Evaluation for Fibrosis

The indices with the highest AUC values for the detection of intermediate and advanced stages of fibrosis (≥3) from higher to lower stages were respectively: FIB4u 0.760 (95% CI: 0.702-0.818), p<0.001; FIB-4 (0.753 (95% CI: 0.694-0.812), p<0.001; GUCI 0.676 (95% CI: 0.611-0.741), p<0.001; and APRI 0.667 (95% CI: 0.601-0.732), p<0.001. FIB-4 and FIB4u indices performed significantly higher AUC values than APRI and GUCI indices (respectively; p=0.001, p=0.003). Threshold values were determined as 0.515 for APRI, 0.365 for GUCI, 1.965 for FIB-4, and 0.456 for FIB4u (Table 8).

The AUC values of indices for detection of advanced stage of fibrosis (≥5) are respectively: FIB-4 0.818 (95% CI: 0.748-0.889), p<0.001; GUCI 0.774 (95% CI: 0.699-0.849), p<0.001; APRI 0.771 (95% CI: 0.696-0.846), p<0.001; and FIB4u 0.770 (95% CI: 0.677-0.862, p<0.001. In the examination, it was seen that the indices were not significantly superior to each other in the diagnosis of advanced stage of fibrosis (p>0.05).

The threshold values for APRI was 0.745, GUCI was 0.905, FIB-4 was 2.080 and FIB4u was 0.459 (Table 8).

The ROC curves of the APRI, GUCI, FIB-4 and FIB4u indices for fibrosis stages ≥3 and ≥5 are shown in Figure 1 and Figure 2.

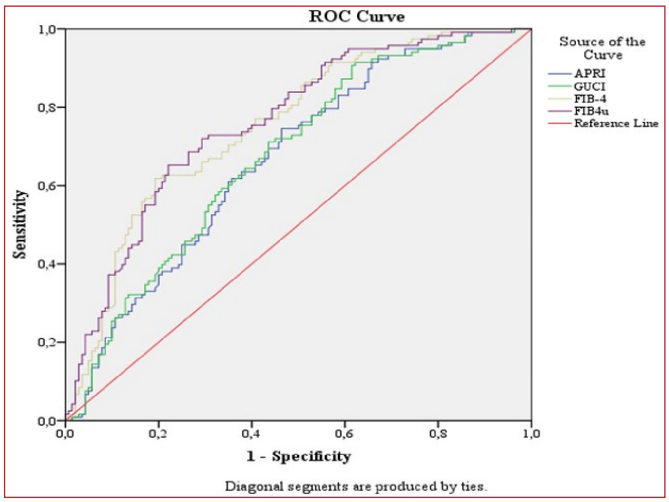

Figure 1. ROC curve for differentiation of fibrosis stage<3 and ≥ 3 distinction

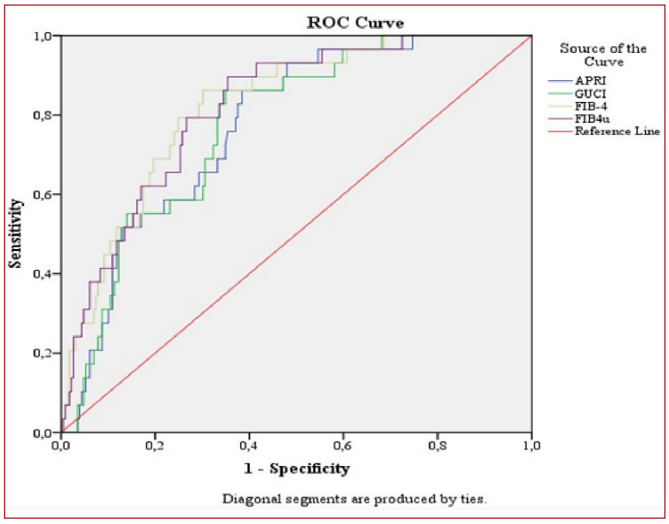

Figure 2. ROC curve for differentiation of fibrosis stage<5 and ≥5

The performances of the indices for fibrosis ≥3 and ≥5 distinctions were compared with each other. FIB-4 and FIB4u were found to be superior to other indices in detecting fibrosis ≥3. Compared to each other, they were not superior to each other. None of them were found to be superior to the other in detecting of fibrosis stage≥5 (Table 9).

Table 8. ROC curve analysis findings of indices for ≥ 3 and ≥ 5 fibrosis distinction

	AUC (%95 CI)	Cut-off value	Sensitivity	Specificity	Positive predictive value	Negative predictive value
APRI						
Fibrosis ≥3	0.667 (0.601-0.732)	0.515	0.737	0.532	0.569	0.708
Fibrosis ≥5	0.771 (0.696-0.846)	0.745	0.862	0.613	0.219	0.972
GUCI						
Fibrosis ≥3	0.676 (0.611-0.741)	0.365	0.915	0.379	0.554	0.841
Fibrosis ≥5	0.774 (0.699-0.849)	0.905	0.862	0.646	0.236	0.974
FIB-4						
Fibrosis ≥3	0.753 (0.694-0.812)	1.965	0.619	0.809	0.703	0.698
Fibrosis ≥5	0.822 (0.752-0.893)	2.080	0.862	0.700	0.266	0.976
FIB4u						
Fibrosis ≥3	0.760 (0.702-0.818)	0.456	0.653	0.773	0.706	0.727
Fibrosis ≥5	0.818 (0.748-0.889)	0.459	0.897	0.643	0.241	0.980

ROC: receiver operating characteristic, AUC: Area Under Curve, CI: confidence interval; APRI: AST / Platelet Ratio Index, GUCI: Goteborg University Cirrhosis Index, FIB-4: Fibrosis 4 İndex, FIB4u: Fibrosis 4 İndex-Ultrasonography,

Table 9. Comparison of the performances of the indices for ≥ 3 and ≥ 5 fibrosis distinction

	Fibrosis ≥3 (p)	Fibrosis ≥5 (p)
APRI - GUCI	0.058	0.581
APRI - FIB-4	0.001	0.159
APRI - FIB4u	0.001	0.280
GUCI - FIB-4	0.003	0.170
GUCI - FIB4u	0.003	0.306
FIB-4 - FIB4u	0.672	0.852

APRI: AST / Platelet Ratio Index, GUCI: Göteborg University Cirrhosis Index, FIB-4: Fibrosis 4 Index, FIB4u: Fibrosis 4 Index-Ultrasonografi.

DISCUSSION

In our study, a positive correlation was found between the age of the patients and the stage of fibrosis. It was consistent with the literature. We did not find a statistically significant difference between gender and fibrosis when we analyzed their relationship. Similarly, in the study of 304 chronic HBV patients by Saglam et al.8, there was a significant correlation between age and fibrosis, but no correlation between gender and fibrosis.

In our study examining the correlation between Ishak fibrosis scores and laboratory results, we discovered that leukocytes, neutrophils, platelets, and albumin values decreased as the Ishak fibrosis score increased. In contrast, the values for urea, AST, ALP, GGT, PTZ, INR, total and direct bilirubin increase simultaneously.

It is known that ALT and AST rise in the blood in liver damage. Nevertheless, the threshold values for determining the extent of damage are unclear. It has been reported in the literature that as fibrosis progresses, AST clearance decreases, and with concurrent mitochondrial injury, AST levels rise significantly more than ALT levels.[9] Similar to our research, we discovered a positive correlation between the stage of fibrosis and AST in other published study.[10]

Gamma-glutamyl transpeptidase is an enzyme found in the microsomes of hepatocytes and gall bladder epithelium. Elevated levels of GGT are observed in liver, gall bladder, and pancreatic disorders. Eminler et al.[11] in a study conducted with 246 HBV and 151 HCV patients in 2014, stated that GGT was found to be significantly higher in patient groups with significant hepatic fibrosis. Saglam et al.[8] also stated in their study that GGT was higher in patients with significant fibrosis. Similar to the studies we mentioned, we found a statistically significant positive correlation between the increase in fibrosis score and the increase in GGT levels.

Studies have reported that with the increase in fibrosis in the liver, there is a decrease in thrombopoietin production in hepatocytes and as a result thrombocytopenia develops.[12] In a study by Iacobellis et al.[13] in 1143 chronic HCV patients, the platelet level threshold value <140,000/mm3, has a high sensitivity in demonstrating cirrhosis. In a study conducted by Aygün et al.[14] with 140 HBV patients, it was stated that the platelet counts were significantly lower in patients with high fibrosis degree compared to those with low fibrosis. Karasu et al.[15] in a study conducted on 519 HBV and 265 HCV patients, excluded patients with splenomegaly and reported that platelet level was negatively correlated with fibrosis stage in chronic hepatitis patients, independent of splenic sequestration. In our study, we found that platelet level was also negatively correlated with fibrosis.

It is known that alkaline phosphatase is significantly increased in biliary tract diseases. Lun-Gen Lu et al.[16] reported that high ALP levels may also be associated with fibrosis in the liver in their study of 200 patients with chronic liver failure. Aygün et al.[14] on the other hand, stated that there was no significant relationship between ALP level and fibrosis staging. In our investigation, a positive correlation was found between the level of ALP and the stage of fibrosis. Although concurrent biliary tract pathology is not observed on scanned US, we cannot make a meaningful generalization because this has not been investigated with more sensitive techniques.

The mean scores of the APRI, GUCI and FIB-4 indices calculated in our study were significantly higher in the patients with high fibrosis scores. In 2003, Chun-Tao Wai et al.[17] reported that the APRI score, which they devised using liver biopsy data and laboratory results of 192 chronic HCV patients, could predict significant fibrosis (≥F3) in 51% of cases and cirrhosis (≥F5) in 88% of cases. In the study, the AUROC value of the APRI score for predicting substantial fibrosis (≥F3) was 0.88 and for predicting cirrhosis (≥F5) was 0.94. Similar results were found for cirrhosis in patients with chronic HBV infection. The AUROC values for the APRI score in considerable fibrosis and cirrhosis were 0.81 and 0.83, respectively, according to a study by Xia Zhu et al.[18] that examined the relationship between liver biopsies and APRI scores in HBV patients. In our study, the AUROC values of the APRI score were found to be 0.66 for F≥3 and 0.77 for F≥5. The AUROC values of the APRI score in our study were found to be lower in demonstrating fibrosis when compared to other studies.

Another index that can be used to predict fibrosis and cirrhosis is GUCI. Islam et al.[19] created the GUCI as a consequence of a study that was carried out in 2004 with 179 chronic HCV patients. In a 2009 study involving 68 chronic HCV patients, Kandemir et al.[20] found that the GUCI score distinguished between stages 3-4 and 1-2 with a high degree of precision. In our study, the AUROC values of the GUCI score were found to be 0.67 for F≥3 and 0.77 for F≥5.

The Fibrosis-4 Index was developed by Sterling et al.[21] in 2006 to predict liver fibrosis in HCV-HIV co-infected patients. The AUROC of the FIB-4 index was found to be 0.76 in estimating fibrosis stage≥4. In the study of Vallet et al.[22] in which they examined liver biopsy results and FIB-4 indices of 847 HCV patients; AUROC values of the FIB-4 index in patients with significant fibrosis (F3-F4) and cirrhosis were reported as 0.85 and 0.91, respectively. In this study, it was reported that the FIB-4 index accurately predicted 847 liver biopsies with a rate of 72.8%. In the study of Xia Zhu et al.[18] in which HBV patients (n=175) were examined the AUROC values for the FIB-4 score in significant fibrosis and cirrhosis were found to be 0.86 and 0.77, respectively. The World Health Organization also recommends the use of the FIB-4 index in the follow-up of chronic HBV patients.[23] In our study, the AUROC value of the FIB-4 index for F≥5 was found to be 0.82, and AUROC values were similar to the studies in the literature.

FIB-4, GUCI and APRI scores were significantly higher in patients with significant liver fibrosis (F≥3). When we performed logistic regression analysis on patients with F≥3 and added ultrasonography findings to the FIB4 index, we created a new model that substantially predicts fibrosis. Although the presence of microlobulation in the liver did not have a statistically significant contribution to the model, it contributed positively to the model. For this reason, we

included microlobulation together with hepatosteatosis in the new model named FIB4u. For the newly developed FIB4u index in patients with F≥3, the mean AUROC value was 0.76. Although we found the most successful AUROC value for FIB4u in predicting F≥3 fibrosis stage, there was no statistically significant difference between the performances of FIB-4 (0.75). In addition the performances of FIB-4 and FIB4u were significantly superior to APRI and GUCI for F≥3.

CONCLUSION

Upon analysis of the data obtained from our investigation, it has been determined that the FIB4u index exhibits promising potential for utilization in the prediction of fibrosis. It demonstrates comparable efficacy to other authorized indices now in use. Nevertheless, in order to ensure widespread utilization, it is imperative that further validation of this approach be conducted using cohorts comprising bigger patient populations. The study is limited by its retrospective design and the limited sample size of patients. In conclusion, it is necessary to conduct bigger prospective studies, incorporating elastography, in order to establish more accurate combined noninvasive indices for the identification of fibrosis.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of Trakya University Faculty of Medicine Scientific Researches Ethics Committee (Date: 10.08.2020, Decision No: 12/10).

Informed Consent

Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

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Author Contributions

All of the authors declare that they have all participated in the design, execution and analysis of the paper and that they have approved the final version.

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