The gamma-glutamyl transferase to platelet ratio for noninvasive evaluation of liver fibrosis in patients with primary biliary cholangitis

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Abstract
The gamma-glutamyl transferase to platelet ratio (GPR) has been reported to be as effective as the aspartate transaminase to platelet ratio index (APRI) and fibrosis index based on the 4 factors (FIB-4) in showing the fibrosis stage in patients with primary biliary cholangitis (PBC). We investigated the effectiveness of GPR in predicting advanced fibrosis and cirrhosis in patients with biopsy-proven untreated PBC. A total of 35 patients with biopsy-proven PBC were included in this study. The biopsy fibrosis stages of all patients at diagnosis were compared using the APRI, FIB-4, and GPR values. The diagnostic accuracy of GPR for detecting advanced fibrosis and cirrhosis was also investigated. The area under the receiver operating characteristic curve (AUROC) of GPR was 0.84, the cutoff point was 4.81, the sensitivity was 0.41, and the specificity was 0.96 for detecting advanced fibrosis. Our study showed that GPR was more sensitive than APRI and FIB-4 in detecting advanced fibrosis in patients with PBC. GPR could be used as an effective noninvasive marker in PBC to show advanced fibrosis at the time of diagnosis.

Abbreviations: ALP = alkaline phosphatase, ALT = alanine aminotransferase, AMA = anti-mitochondrial antibody, APRI = aspartate transaminase to platelet ratio index, AST = aspartate aminotransferase, AUROC = area under the receiver operating characteristic curve, FIB-4 = fibrosis index based on the 4 factors, GGT = gamma-glutamyl transferase, GPR = gamma-glutamyl transferase to platelet ratio, PBC = primary biliary cholangitis, ROC = receiver operation characteristic, UDCA = ursodeoxycholic acid.

Keywords: gamma-glutamyl transferase to platelet ratio, liver fibrosis, primary biliary cholangitis

1. Introduction and Objectives
Primary biliary cholangitis (PBC) is an autoimmune disorder characterized by the excretion of the intrahepatic bile ducts, often leading to cirrhosis and liver failure.[1,2] Although the underlying causes remain unclear, genetic predisposition, various nucleotide polymorphisms, and environmental factors are thought to be responsible.[3,4] The course of PBC can progress to chronic cholestasis, portal inflammation, fibrosis, cirrhosis, liver failure, and even liver cancer, which develops as a result of damage to the small and medium-sized bile ducts. At least 2 of the following 3 criteria must be met for the diagnosis: serum anti-mitochondrial antibody (AMA) or AMA-M2 positivity, unexplained elevation of alkaline phosphatase (ALP) at least 1.5 times higher than normal that persists for 24 weeks, cholangitis not characterized by suppuration, and interlobular bile duct damage in liver biopsy. In chronic liver disease, the degree of hepatic fibrosis at diagnosis provides information regarding the stage of the disease, response to treatment, and prognosis.[5] Although a liver biopsy is not an absolute requirement for the diagnosis of PBC, it is the gold standard for the evaluation of hepatic fibrosis. However, diagnostic methods for noninvasive hepatic fibrosis assessment have come to the fore in recent years owing to the invasiveness, high cost, and serious side effects of liver biopsy.

The efficacy of noninvasive methods, such as serum assays and imaging methods, has been tested. In particular, the aspartate transaminase (AST) to platelet ratio index (APRI) and the fibrosis index based on the 4 factors (FIB-4) are becoming increasingly precise, as they are simple, inexpensive, reliable, and easily accessible. APRI has been associated with histological progression in liver biopsies in patients with PBC,[6,7] and APRI and FIB-4 are associated with advanced fibrosis in nonalcoholic fatty liver disease.[8] The gamma-glutamyl transpeptidase to platelet ratio (GPR), developed in 2015, is as effective as APRI and FIB-4.
in detecting advanced fibrosis and cirrhosis in patients with chronic hepatitis B.\textsuperscript{9,10} Gamma-glutamyl transferase (GGT) is located on the capillary surface of liver cells and on the membrane side of bile duct epithelial cells. GGT levels increase in bile secretion obstruction and bile duct damage, thus GGT is helpful in the diagnosis of cholestatic liver diseases.\textsuperscript{11} The European Association for the Study of the Liver guidelines recommend its use for the diagnosis of PBC. It has also been associated with the prognosis of PBC.\textsuperscript{12,13} There is no study in the literature investigating the predictive power of GPR for fibrosis in PBC. In our study, we investigated the relationship between GPR, a GGT-based fibrosis assessment marker, and liver histology at the time of diagnosis, and its power to detect advanced fibrosis and cirrhosis in PBC.

2. Materials and methods

2.1. Study population

The files of 154 patients diagnosed with PBC in the hospital information operating system between January 2008 and December 2020 at Ondokuz Mayıs University Faculty of Medicine were retrospectively reviewed. Those who did not meet the diagnostic criteria for PBC (n = 56), those who had another chronic liver disease accompanying PBC (7 autoimmune hepatitis, 3 overlap syndromes, 2 nonalcoholic steatohepatitis, 4 others, n = 16), and those who did not undergo a liver biopsy at the time of diagnosis (n = 47) were excluded from the study. A total of 35 patients who met the inclusion criteria were enrolled in the study (Fig. 1).

The study protocol was approved by the ethics committee of the Ondokuz Mayıs University Faculty of Medicine (decision no: 522, date: 02.12.2021), and the procedures were in accordance with the Helsinki Declaration of 1973, as revised in 1983.

2.2. Clinical and laboratory assessments

Demographic data (age, sex) and laboratory values obtained during the biopsy (platelet, alanine aminotransferase, aspartate aminotransferase (AST), GGT, ALP, total bilirubin, and AMA and anti-nuclear antibody were retrospectively recorded. Blood tests were performed on the day of the biopsy or at most 1 week before. All patients were treatment naive. The APRI, FIB-4, and GPR values were calculated using the formulas given below: Liver biopsy was performed with a 16g needle under ultrasonographic guidance, and a minimum of 1.5 cm length liver tissue was obtained for pathological examination. Pathological evaluation was performed using preparations containing at least 5 portal areas. Fibrosis was classified according to the Scheuer classification as follows: F0, no fibrosis; F1, portal fibrosis; F2, portal fibrosis with few septa; F3, numerous septa without cirrhosis; F4, cirrhosis.\textsuperscript{14} In addition, patients were divided into 2 groups: early stage (F1 and F2) and advanced-stage (F3 and F4) fibrosis. Laboratory values, APRI, FIB-4, and GPR were compared between the groups with early and advanced fibrosis. In addition, ROC analysis
Table 1

| The demographic data and laboratory values of the patients. |
|------------------------------------------------------------|
| (mean ± SD) | (min-max) |
| Age (yr) | 49.6 ± 10.88 | (29–69) |
| (n) | (%) |
| Female | 33 | 94.3 |
| Male | 2 | 5.7 |
| AMA+ | 32 | 91.4 |
| AMA−, AMA+ | 3 | 8.6 |
| AST (U/L) | 50.8 ± 32.7 | (18.7–160) |
| ALT (U/L) | 50.6 ± 26.2 | (13.9–99) |
| ALP (U/L) | 325.5 ± 236.9 | (81–1137) |
| GGT (U/L) | 199.9 ± 136 | (18.1–578.6) |
| Bilirubin (mg/dL) | 1 ± 1.7 | (0.25–10.1) |
| Platelet (×10⁹/L) | 242.5 ± 98.7 | (84–467) |
| APRI | 0.56 ± 0.7 | (0.1–4.1) |
| FIB-4 | 1.81 ± 1.48 | (0.39–7.3) |
| GPR | 3 ± 3.67 | (0.26–19.1) |

ALP = alkaline phosphatase, ALT = alanine aminotransferase, AMA = anti-mitochondrial antibody, AMA = anti-nuclear antibody, APRI = aspartate transaminase to platelet ratio index, AST = aspartate aminotransferase, FIB-4 = fibrosis index based on the 4 factors, GGT = gamma-glutamyl transferase, GPR = gamma-glutamyl transferase to platelet ratio, SD = standard deviation.

was performed for APRI, FIB-4, and GPR to determine their effectiveness in detecting advanced fibrosis.

2.3. Formulas

APRI : \( \frac{[\text{AST (upper bound of the normal AST range)} \times 100]}{\text{platelet.}} \)

FIB-4 : Age (years) × AST (U/L) / (platelets \( \times 10^9/L \) × [ALT (U/L)] \( \times 100 \))

GPR : \( \frac{[\text{GGT (upper bound of the normal GGT range)} \times 100]}{\text{platelet.}} \)

Upper bound of the normal AST range : 32 U/L

Upper bound of the normal GGT range : 40 U/L

2.4. Data analysis and statistics

In descriptive statistics, mean, standard deviation, median value, and 25% to 75% values were used for numerical variables, and numbers and percentages were used for categorical variables. The independent samples t test was used to analyze differences between 2 groups of variables with parametric distribution, the Mann–Whitney U test was used to analyze differences between 2 groups of variables with non-parametric distribution, and the Kruskal–Wallis test was used to analyze differences between more than 2 groups of variables with non-parametric distribution. Pearson chi-square test was used for intergroup comparisons of categorical variables. The area under the receiver operating characteristic curve (AUROC) was used to detect the APRI, FIB-4, and GPR sensitivity, specificity, and optimal cutoff value to show the fibrosis stage. \( P < .05 \) was considered statistically significant.

3. Results

Among the 35 patients with biopsy-proven PBC who were treatment-naive, 33 were female (94.3%) and 2 were male (5.7%). Of the patients, 32 (91.4%) had positive AMA, 3 (8.6%) had negative AMA and positive anti-nuclear antibody. The general information and laboratory values of the patients are presented in Table 1.

Table 2

| The biopsy scores of the patients. |
|-----------------------------------|
| Fibrosis stage | n (%) |
| I | 14 (40) |
| II | 9 (25.7) |
| III | 6 (17.1) |
| IV | 6 (17.1) |
| Total | 35 |

According to the Scheuer fibrosis scoring system, 14 patients (40%) were stage 1, 9 patients were stage 2 (25.7%), 6 patients were stage 3 (17.1%), 6 patients were stage 4 (17.1%). The distribution of the patients according to their fibrosis scores is shown in Table 2.

In the advanced-stage fibrosis group, GGT, platelet, FIB-4, and GPR values were found to be high and statistically significant, while platelet count was low. There were no significant differences between the 2 groups in age, sex, AST, alanine aminotransferase, ALP, total bilirubin, and APRI values. The data of the 2 groups in which the patients were divided into early stage (F1 and F2) and advanced stage (F3 and F4) fibrosis groups are shown in Table 3.

The sensitivity and specificity of APRI were 33% and 96%, respectively, and the AUROC was 0.71. The sensitivity and specificity of FIB-4 were 33% and 96%, respectively, and the AUROC value was 0.82 (\( P < .02 \)). The sensitivity and specificity of GPR were 41% and 96%, respectively, and the AUROC value was 0.84 (\( P < .01 \)). The diagnostic accuracy of the fibrosis scores in determining advanced fibrosis is shown in Table 4. The ROC analysis of APRI, FIB-4, and GPR for the detection of early and advanced stages of hepatic fibrosis is shown in Figure 2.

4. Discussion

PBC is a slowly progressive, immune-mediated, cholestatic liver disease with destruction of intrahepatic bile ducts, mostly diagnosed in the 5th or 6th decade, and is frequently seen in females, and AMA is positive in the majority of cases.[15] In our study, the sex distribution, mean age, and rate of AMA positivity were consistent with those reported in the literature. The clinical features of PBC are heterogeneous, and the course of the disease is highly variable. While some patients develop cirrhosis within a few years, others may follow a rather slow course.[12,13] Early disease stages are associated with a good prognosis; however, the condition is irreversible in cirrhotic patients, and the risk of decompensation and hepatocellular carcinoma is increased.[14,17] Ursodeoxycholic acid (UDCA) is the only treatment that changes the course of PBC. The best response to UDCA treatment was observed in patients with early-stage fibrosis. UDCA treatment did not reduce PBC-related morbidity, mortality, or transplantation-free survival in patients with advanced fibrosis. Obeticholic acid is also approved as an adjunct to UDCA in patients unresponsive to UDCA or as monotherapy in patients who have intolerance for UDCA.[12]

The presence of advanced fibrosis at the time of diagnosis is not only associated with reduced transplant-free survival and poor prognosis but also with poor prognosis despite the biochemical response to UDCA (normalized ALP levels).[17] Therefore, understanding the stage of liver fibrosis in PBC is important to predict the course of the disease. Although biopsy is the gold standard for the evaluation of liver fibrosis, it has been substituted by noninvasive methods for the assessment of hepatic fibrosis due to its invasiveness, inaccessibility, and risks. Among these, APRI, FIB-4, and elastography are widely used methods.[7,14,19] The relationship between PBC and many fibrosis markers has been evaluated.[7,20,21] In a study involving 107 patients diagnosed with
PBC, erythrocyte distribution width, FIB-4, albumin, and platelet values were found to be associated with fibrosis, among which FIB-4 had the highest sensitivity and specificity for distinguishing histological severity. Joshita et al demonstrated that APRI is associated with histological grade and disease progression in patients with PBC. Ölmez et al showed that APRI, FIB-4, and AST values in PBC patients were significantly higher in the early
(F1-2) and advanced stage (F3-4) fibrosis groups according to the Scheuer scoring system in their study.[18] However, in a study conducted by Corpechot et al, elastography was found to be superior to APRI and FIB-4 in detecting advanced fibrosis and cirrhosis.[21] Elastography is not available in every clinic and requires additional cost and time compared with serum-based noninvasive fibrosis markers. Therefore, it is clear that there is still a need for inexpensive and easily calculable fibrosis prediction markers.

In our study, GPR, a parameter that has not been previously studied as a noninvasive fibrosis detection method in patients with PBC, was evaluated. GPR was found to be effective in predicting advanced fibrosis and cirrhosis in chronic hepatitis B patients.[9,10] Lemoine et al demonstrated that GPR was more effective in predicting advanced fibrosis and cirrhosis than APRI and FIB-4 in patients with hepatitis B in sub-Saharan Africa.[9] Ren et al showed that GPR was more effective than FIB-4 in detecting fibrosis in patients with hepatitis B, whereas FIB-4 was more effective in detecting cirrhosis.[10] In a recent study, the S-index calculated with a special formula consisting of GGT, platelets, and albumin was found to be significantly higher in patients with PBC in the advanced fibrosis group (F3-4) according to the Scheuer system.[19]

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