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Development and evaluation of a novel score for prediction of large oesophageal varices in patients with hepatitis c virus-induced liver cirrhosis

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ABSTRACT

Objective: Variceal bleeding is one of the most common life-threatening complications of liver cirrhosis. This study aimed to develop and evaluate a predictive score, named **P**latelet count, **A**lpha fetoprotein (AFP) and **P**rothrombin-INR (PAP) for the prediction of large oesophageal varices and to compare PAP score with eight common liver fibrosis scores (AAR, APRI, GUCI, BRC score, Fibro-Alfa, FIB4, Lok and Fibro-Q) in patients with hepatitis C virus (HCV) induced liver cirrhosis.

Methods: A total of 277 patients with HCV-induced liver cirrhosis were evaluated by upper gastrointestinal endoscopy for presence of varices. Liver biochemical profile, complete blood count, prothrombin time and AFP were estimated. Stepwise linear discriminant analysis and area under receiver-operating characteristic curves (AUCs) were used to create a predictive score (PAP score) comprising **p**latelet count, **A**FP and **p**rothrombin-INR.

Results: PAP score predicts large oesophageal varices in patients with HCV-induced liver cirrhosis with AUC of 0.85. The optimum cut-off for predicting large oesophageal varices using ROC curve analysis was 0.27. At this point the PAP score had 77% sensitivity, 86% specificity, 94% negative predictive value and 84% efficiency. The diagnostic performances (AUC) of eight common liver fibrosis scores were 0.58 for the AAR score, 0.63 for APRI, 0.66 for GUCI, 0.68 for BRC, 0.72 for Fibro-Alfa, 0.70 for FIB4, 0.72 for Lok and 0.77 for Fibro-Q.

Conclusion: PAP scores a non-invasive, inexpensive and simple score that could predict the presence of large oesophageal varices reducing the need of endoscopy. The PAP score has a superior AUC score than other scores, suggesting improved clinical value.

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KEYWORDS
Liver cirrhosis; large oesophageal varices; blood markers; score

Introduction

Hepatitis C virus (HCV) is one of the major aetiologies that cause liver cirrhosis [1]. Portal hypertension is the primary complication of liver cirrhosis and induces development of oesophageal varices which is the most common lethal complications of cirrhosis [2]. The detection rate of oesophageal varices is about 16% in patients with hepatitis C with bridging fibrosis. Gastroesophageal varices are detected in about 50% of patients with liver cirrhosis and its detection rate correlates with the severity of liver disease (40% of Child A patients and 85% of Child C patients) [3,4]. Endoscopic screening is the gold standard for the diagnosis of oesophageal varices. However, upper GI endoscopy is invasive and costly thus there is a need for a noninvasive, simple, inexpensive, accurate and quick method with no additional burden to patients for oesophageal varices screening [5]. The predictive methods of large oesophageal varices have been focusing on laboratory/or clinical parameters. The laboratory routine blood markers include aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio (AAR), platelet count, aspartate to platelet ratio index (APRI) and albumin level. The clinical noninvasive predictive imaging includes spleen diameter, size of right liver lobe, and portal vein diameter [6–8]. However, all these predictive methods showed low diagnostic performances. In the present study, we aimed to develop a new score for prediction of large oesophageal varices and evaluate its diagnostic performance compared to common liver fibrosis scores in diagnosing large oesophageal varices in patients with HCV-induced liver cirrhosis. Common serum fibrosis scores include AAR [9], APRI [10], Gotebörg University Cirrhosis Index (GUCI) [11], Biotechnology Research Center (BRC) score [12], Fibro-Alfa [13], FIB4 [14], Lok [15] and Fibro-Q [16].

Materials and methods

A total of 277 consecutive patients with HCV-induced cirrhosis recruited from Tropical Medicine Department, Mansoura University, Mansoura, Egypt during the period from January 2014 to December 2015 were included. A full medical history was taken. All patients underwent

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a complete physical and routine laboratory examination. Laboratory tests were done in Clinical Pathology Department, Faculty of Medicine, Al-Azhar University, New Damietta, Egypt. Diagnosis of cirrhosis was based on clinical, laboratory and ultra-sonographic findings. All patients were tested negative for HBsAg (Dia.Pro, Milan, Italy) and were tested positive for anti-HCV antibodies (Biomedica, Sorin, Italy). Patients were then confirmed for the presence of HCV-RNA using quantitative polymerase chain reaction assay (COBAS Ampliprep/COBAS TaqMan, Roche Diagnostics, Pleasanton, USA). All patients subjected to upper gastrointestinal endoscopy using forward viewing fibreoptic endoscopy (Olympus Q 30-Japan). If oesophageal varices were present, their size was graded as I-IV, using the Paquet grading system [17]. Patients were classified to either as having large oesophageal varices (grade III-IV) or as not having these (no varices or grade I-II). Patients with history of upper gastrointestinal bleeding, patients treated with beta-blockers or other vasoactive drugs, patients with any coexistent illness or infection that could influence platelet count and splenomegaly causes rather than cirrhotic portal hypertension were excluded.

Blood samples were collected from all patients with cirrhotic liver, a portion of the blood was mixed immediately with potassium ethylene diaminetetraacetic acid (K-EDTA) for complete blood count and another portion was mixed with a citrate solution for prothrombin-INR (international normalised ratio, INR). Sera were separated from the rest of blood samples and tested for liver profile. The blood tests included liver profile (transaminases, bilirubin, and serum albumin), complete blood count, prothrombin time and AFP. Liver profile was measured on an automated biochemistry analyzer (Beckman Coulter AU480). Complete blood pictures including platelets counting were determined by KX-21 Sysmex automated hematology analyzer (Sysmex Corporation, Japan). AFP level was measured by chemiluminescence, with Immulite (1000), AFP kit (Diagnostic Products Corporation; Los Angeles, CA, USA). The study protocol conforms to the ethical guidelines of the 1975 Declaration of the Helsinki.

Statistical analysis was as follows. Continuous variables are presented as mean ± SD. Statistical differences were tested using Student's t-test or nonparametric Mann–Whitney test. Categorical values were compared using chi-square test. Association of the candidate variables with large oesophageal varices was assessed using univariate analysis. Significant associated variables were further included in a stepwise logistic regression model to evaluate their independent contribution to the presence of large oesophageal varices. Predictive contribution to large oesophageal varices from those candidate variables was further determined by receiver operating characteristic (ROC) curves. Predictive models were then validated by the mean areas under the ROC curves (AUC). A cut-off value serving as a diagnostic criterion (presence or absence of large oesophageal varices) was optimised for the best predictive function. The multivariate logistic regression was analysed to estimate the odds ratios (ORs) of the relations between noninvasive scores and large oesophageal varices.

Results

A total of 277 patients with HCV-induced cirrhotic were included in the study, 167 (60%) male and 117 (40%) females with mean age 53.2 ± 6.8 years. Endoscopic examination revealed the presence of oesophageal varices of different grades. Overall, 114 patients (41.1%) had no oesophageal varices, 111 patients (40.1%) had grade I & II and 52 patients (18.8%) had grade III & IV (large) varices. The laboratory blood markers of patients with HCV-induced liver cirrhosis according to presence or absence of large oesophageal varices on univariate analysis are summarised in Table 1. ALT, albumin, total bilirubin, prothrombin-INR, platelet count and quantitative PCR were independent variables for prediction of large oesophageal varices ($p < 0.05$). The median of HCV-RNA

Table 1. Relationship of laboratory parameters with the presence or absence of large oesophageal varices on univariate analysis.

Variables	No or Small varices ($n = 225$)	Large varices ($n = 52$)	*P* value
Age (years)[a]	53.3 ± 6.4	52.4 ± 4.9	0.356
Liver profile			
AST (U/L)[a]	73.4 ± 36.1	65.2 ± 34.6	0.144
ALT (U/L)[a]	66.5 ± 39.4	50.8 ± 28.2	0.007
Albumin (g/L)	37.4 ± 5.14	35.3 ± 5.7	0.012
Total bilirubin (mg/dL)[a]	1.02 ± 0.53	1.4 ± 0.76	0.0001
AFP (U/L)	17.4 ± 1.7	25.6 ± 3.1	0.0001
Hematology parameters			
Hemoglobin (g/L)	134 ± 17	132 ± 15	0.436
Prothrombin-INR	1.2 ± 0.16	1.3 ± 0.25	0.0001
Platelet count (10^9/L)[a]	113 ± 45	71 ± 33	0.0001
Virology markers			
Positive HCV-antibody (no; %)	225 (100%)	52 (100%)	
Positive HBV surface antigen (no; %)	0 (0.0%)	0 (0.0%)	
Quantitative PCR (IU ml X10^3)	106.2 ± 163.1	383.0 ± 462.8	0.007

[a]References values: Aspartate aminotransferase (AST) > 40 U/L; alanine aminotransferase (ALT) > 45 U/L; albumin 38–54 g/L; total bilirubin > 1 mg/dl; international normalised ratio (INR) 1; alkaline phosphatase (ALP) 22–92 U/L; platelet count 150–400 × 10^9/L; alpha fetoprotein (AFP) > 10 (U/L).

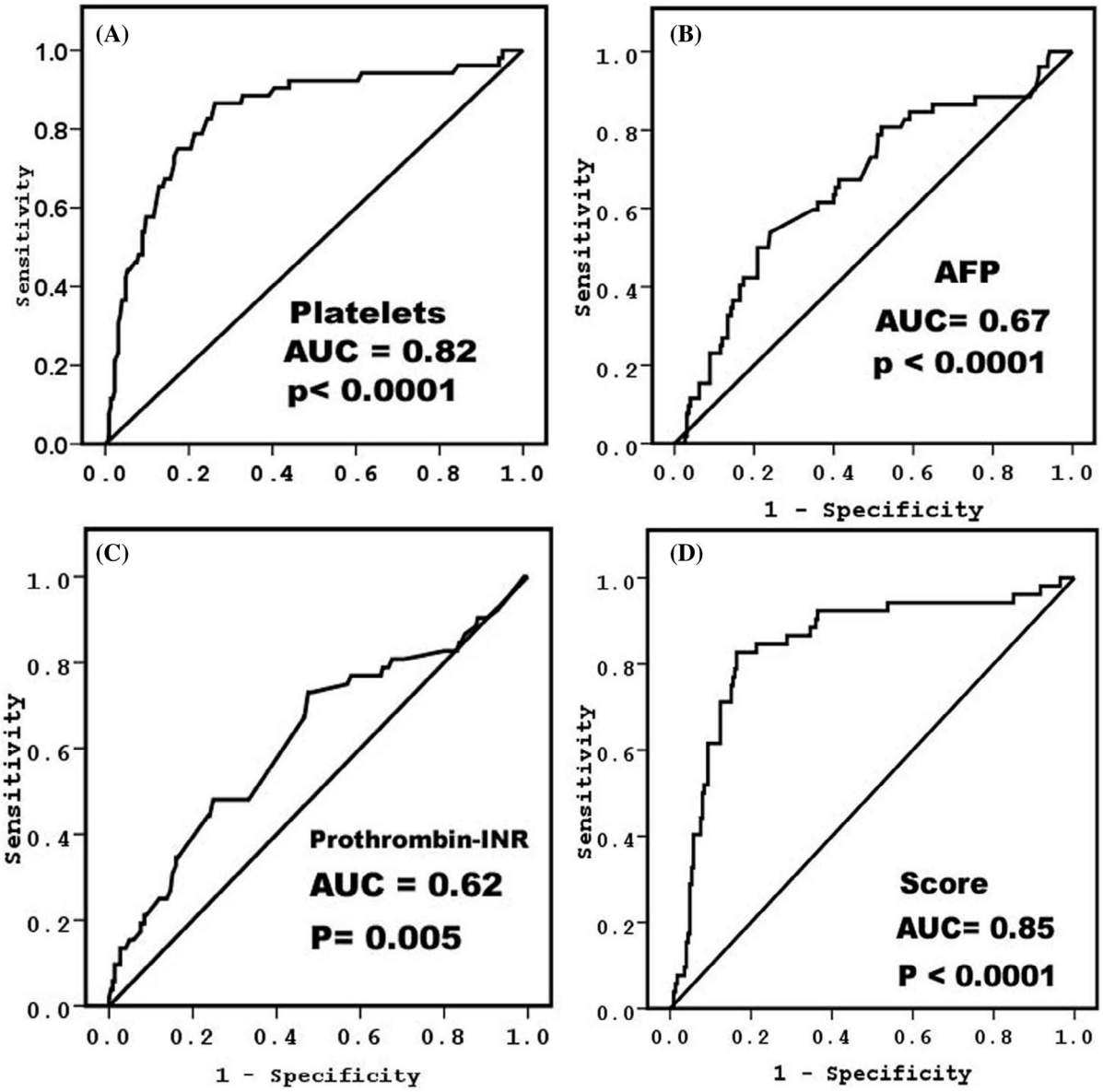

Figure 1. ROC curves of single markers and novel score for discriminated large oesophageal varices among patients with liver cirrhosis. Areas under the curve (AUC) were 0.82, 0.67, 0.62 and 0.85 for platelet count, AFP, prothrombin-INR and novel PAP score; respectively.

levels (IU ml X10^3) was 396.5 and 184.1 for no or small varices and large varices, respectively.

The diagnostic performances of biochemical markers using AUC were as follows. The areas under the ROC curves (95% CI) of platelet count, AFP and prothrombin-INR for discriminating liver cirrhosis patients with large oesophageal varices were 0.82 (0.76–0.89), 0.67 (0.1–0.76), and 0.62 (0.54–0.71), respectively. Platelet count was the most efficient index among other markers with area under ROC curve being 0.82 (Figure 1(A)–(C)).

The performance characteristics of novel score was as follows. The stepwise linear discriminant analysis selected a novel noninvasive index for predicting large oesophageal varices based on platelet count, AFP and prothrombin-INR; named PAP score. PAP Score = (0.038 (numeric constant) + INR × 0.383 + AFP (IU ml)$^{-1}$ × 0.002) – (platelet count (×10^9)$^{-1}$ X 0.003). Where 0.038 is a numeric constant. PAP-score predicts large esophageal with AUC (95% CI) 0.85 (0.78-0.91) (Figure 1(D)). The best cut-off (0.27) was chosen for predicting large oesophageal varices using ROC curve. Using this cut-off, PAP score had 77% sensitivity, 86% specificity, 56% positive predictive value, 94% negative predictive value and efficiency of 84% in predicting large oesophageal varices.

A comparison of diagnostic performances of PAP score and AAR, APRI, GUCI, BRC, Fibro-Alfa, FIB4, Lok, and Fibro-Q using AUC was as follows. The mean ± SD and AUC data of the PAP score and eight common liver fibrosis scores, and so the diagnostic performances of scores were compared to predict large oesophageal varices (Table 2; Figure 2). The AUC of five indices overlapped with that of the PAP score and so was compared in a multivariate logistic regression to determine independence and rank value. This showed that the PAP Score was the

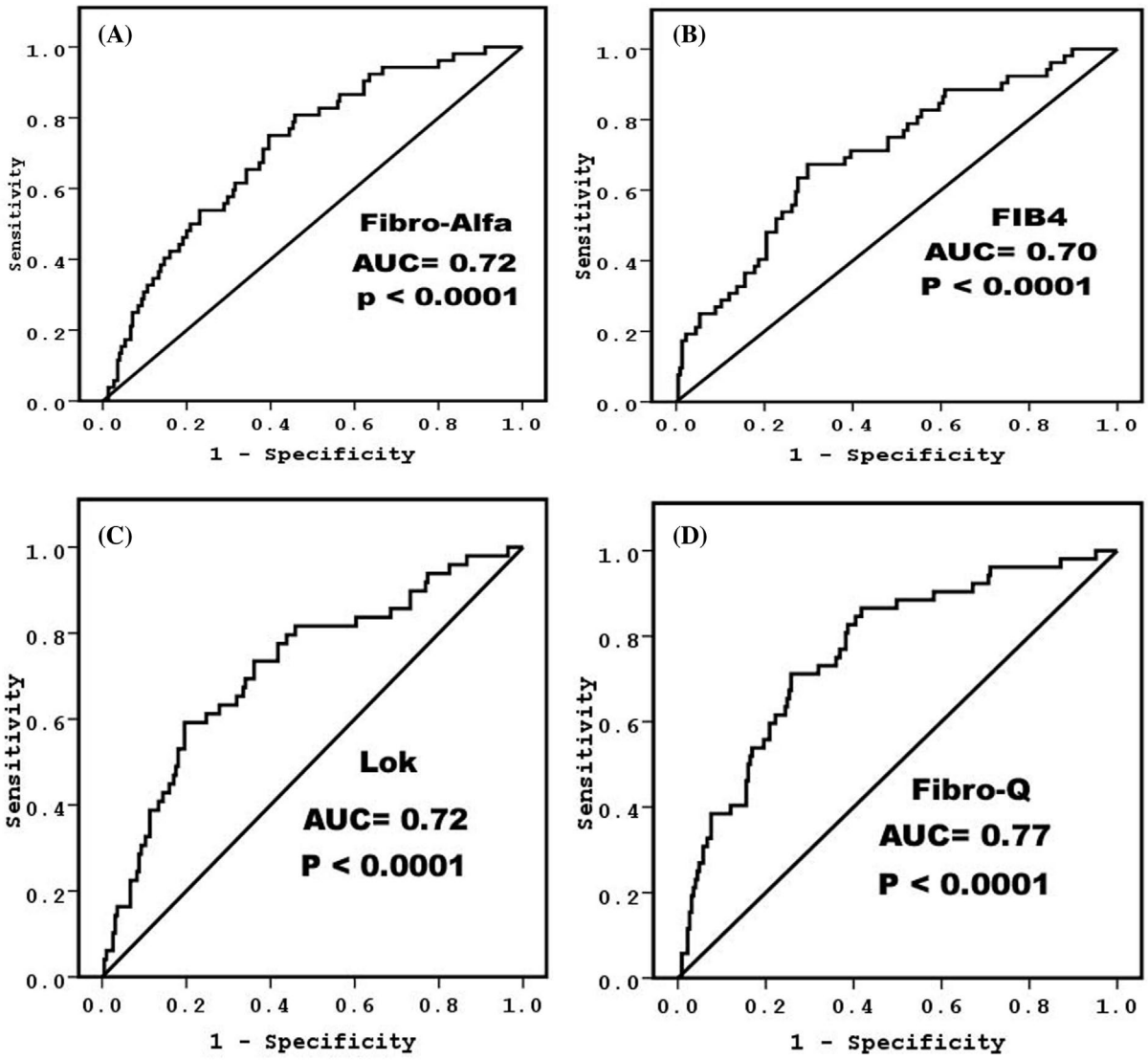

Figure 2. ROC curves of Fibro-Alfa, FIB4, Lok and Fibro-Q for discriminates large oesophageal varices among patients with liver cirrhosis. Areas under the curve (AUC) were 0.72, 0.70, 0.72 and 0.77 for Fibro-Alfa, FIB4, Lok and Fibro-Q; respectively.

most efficient index with an odds ratio (95% CI) for large vs. small varices of 10.8 (4.8–15.6)($p < 0.001$), exceeding that of the FIB-4 (0.93 [0.87–1.11], $p = 0.414$), Fibro-Alfa (1.03 [0.46–2.67], $p = 0.955$), Lok (1.29 [0.73–2.28], $p = 0.387$) and Fibro-Q scores (2.5 [0.68–2.81] $p = 0.168$).

Discussion

Non-invasive methods to diagnose large esophageal varices could decrease the number of endoscopies. In the present study, we developed a predictive score for large esophageal varices based on three markers: **p**latelet count, **A**FP and **p**rothrombin-INR. In chronic hepatitis C, thrombocytopenia may result from bone marrow inhibition, reduction of thrombopoietin production, autoimmune disease. Clinical variables as age, gender, severity of liver disease and HCV-RNA levels could affect the severity of thrombocytopenia. [18] The increase in prothrombin time is due to the low synthesis of coagulation factors (I, II, V, VII and X) which are synthetized in the liver [19]. The level of AFP, the third marker in our score increases in patients with liver cirrhosis due to the re-expression of the related gene, which is usually suppressed in adult subjects [20]. ROC curves can be used to compare several common liver fibrosis scores for diagnosis of large oesophageal varices. Thus ROC analysis has become an important method for the evaluation of diagnostic blood markers for large oesophageal varices [21]. The diagnostic accuracy of ROC is generally defined as useful if area under the curve (AUC) is > 0.7 and defined as excellent if AUC is between 0.8–0.9 [22]. Several authors evaluated the diagnostic performance of common single or combined liver fibrosis markers in diagnosing large oesophageal varices [23]. AST/ALT ratio gave 0.79 AUC with 68% sensitivity, 77% specificity 41% PPV and 92% NPV. APRI demonstrated 65% sensitivity, 73% specificity, 87% PPV, 43% NPV [24]. A platelet count at 68 x 10^9/L cut-off and splenomegaly had 71% vs. 75% sensitivity and 73% vs. 58% specificity for discrimination of large EV [25]. The AUC of hyaluronic acid was 0.92 for

Table 2. Level and area under ROC of PAP score and eight common liver fibrosis scores for detection small and large oesophageal varices.

	Small varices	Large varices		AUC
Variables	*n* = 225	*n* = 52	*P* value	(95% CI)
AAR	1.2 ± 0.48	1.35 ± 0.51	0.073	0.58 (0.49–0.67)
APRI	1.9 ± 1.48	2.64 ± 1.84	0.002	0.63 (0.54–0.72)
GUCI	2.4 ± 1.8	3.4 ± 2.3	<0.0001	0.66 (0.57–0.74)
BRC	15.8 ± 10.6	19.8 ± 9.3	0.014	0.68 (0.61–0.77)
FIB-4	5.2 ± 3.6	7.8 ± 4.5	<0.0001	0.70 (0.62–0.78)
Fibro-Alfa	1.6 ± 0.30	1.8 ± 0.29	<0.0001	0.72 (0.62–0.78)
Lok score	0.26 ± 0.47	0.54 ± 0.32	<0.0001	0.72 (0.62–0.78)
Fibro-Q	0.69 ± 0.50	1.28 ± 1.57	<0.0001	0.77 (0.64–0.79)
PAP score	1.2 ± 0.63	2.9 ± 1.2	<0.0001	0.85 (0.78–0.91)

Abbreviations: AAR = aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio; APRI = aspartate to platelet ratio index; GUCI = Goteborg University Cirrhosis Index; BRC = Biotechnology Research Center; CI = Confidence Interval.

discrimination large oesophageal varices. The sensitivity of hyaluronic acid, specificity, PPV, NPV and diagnostic accuracy was 94, 78, 89, 88 and 88%, respectively [26]. No single marker was found to offer full diagnostic power for large oesophageal varices; however, a combination of several markers improves the diagnostic accuracy. In the present study, a novel PAP score was developed and evaluated for prediction of large oesophageal varices. PAP score had moderate diagnostic accuracy with an AUC of 0.85 for prediction of large oesophageal varices, 77% sensitivity and 86% specificity, 94% negative predictive value. Fibro-Test had the highest diagnostic power with AUC of 0.77 compared with 0.64 and 0.68 for platelet count and Child-Pugh score; respectively [27]. The combination of Lok index and Forns' index had 0.80 AUC with 90% NPV to exclude large OV or small OV [28]. Using a platelet count/spleen diameter ratio had 73% NPV and 74% PPV [29]. A score for the prediction of large oesophageal varices based on hemoglobin level, portal vein diameter and the ratio of platelet count/spleen diameter achieved 77.8 and 72% of diagnostic sensitivity and specificity, respectively [30]. In the present study, AUC of AAR, APRI, GUCI, BRC score, Fibro-Alfa, FIB4, Lok and Fibro-Q scores were 0.58, 0.63, 0.66, 0.68, 0.72, 0.70, 0.72 and 0.77, respectively, for the prediction of large varices. On the other hand, other studies reported similar AUC of APRI, AAR, FIB-4, Lok, and Forns scores for the prediction of large varices (0.73, 0.75, 0.71, 0.73, and 0.65; respectively). APRI, AAR, FIB-4, Lok, and Forns scores had low to moderate diagnostic power in detecting large varices in patients with liver cirrhosis [31]. However, no information exists, regarding the use of GUCI, BRC score, Fibro-Alfa, Fibro-Q scores in prediction of large varices in patients with liver cirrhosis. Further multicentre studies are needed to validate the results of this study.

This study represents an advances in biomedical science because it shows that the PAP score (which requires only routine laboratory tests) could be used for prediction of large oesophageal varices or as additional marker in large oesophageal varices surveillance program in patients with HCV-induced liver cirrhosis and might reduce the need for using endoscopy.

Summary table

What is known about this subject

Variceal bleeding is the most common life-threatening complication of liver cirrhosis

Endoscopic screening is the gold standard for the diagnosis of oesophageal varices

Noninvasive method focusing on blood markers and imaging is common

What this paper adds

Development of a novel score (PAP score) for diagnosis of large oesophageal varices

PAP Score was the most efficient index among eight liver fibrosis scores with area under ROC curve of 0.85

Disclosure statement

No potential conflict of interest was reported by the authors.

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