Clinical Utility of Simple Fibrosis Markers in Prediction of Oesophageal Varices in Chronic Hepatitis C Patients with Advanced Fibrosis

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Abstract

Background and Aims: Oesophageal varices (OV) are the most common complication of liver cirrhosis. However, no available data exist on the prevalence and the laboratory predictors of OV in chronic hepatitis C (CHC) Egyptian patients with bridging fibrosis or cirrhosis. Therefore, we aimed to investigate the frequency of OV in these patients and to assess whether simple non-invasive serum fibrosis markers help in screening endoscopy for detection of OV in these patients.

Patients and Methods: Two hundred CHC treatment naïve patients (116 stage 3: bridging fibrosis and 84 stage 4: cirrhosis) were enrolled in the study. All patients had liver biopsy examined by a single histopathologist using Desmet et al. staging and grading system. Non-invasive fibrosis markers (platelet count, AST/ALT ratio, APRI, Fib 4 index and BARD score) were calculated for all of them. All patients underwent screening upper gastrointestinal endoscopy for detection of OV.

Results: The overall frequency of OV was 100/200 (50%). Forty-two patients (36.2%) of stage 3 had OV. While, Fifty-eight (69%) of patients of stage 4 had OV. Patients with OV had significantly lower platelet count and albumin and higher AST, ALT, APRI. ROC analysis revealed an AUC of 0.351, 0.382, 0.624 for platelets, albumin and APRI respectively.

Conclusions: A high frequency of OV in CHC even before histological detection of cirrhosis indicates that liver biopsy may underestimate the actual prevalence of liver cirrhosis and portal hypertension in these patients. None of the tested serum fibrosis markers can reliably help in screening for diagnosis of OV in CHC patients with stage 3 or 4 fibrosis.

Key Words: *Chronic hepatitis C – Bridging fibrosis – Liver cirrhosis – Oesophageal varices.*

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Introduction

LIVER fibrosis is the main determinant of hepatitis C virus-related morbidity and mortality [1]. In addition, the stage of fibrosis is prognostic and provides information on the likelihood of disease progression and response to treatment [2]. The onset of liver fibrosis is usually insidious, and most of the related morbidity and mortality occur after development of cirrhosis [3]. Natural history studies suggest that hepatitis C infection progresses to cirrhosis in 20% of cases [4].

Portal hypertension results from progressive fibrotic remodeling of the liver, which increases the resistance to hepatic sinusoidal blood flow. Increased portal venous pressure causes oesophageal and gastric varices, which contribute substantially to cirrhosis-related morbidity and mortality [5].

Oesophageal varices (OV) are present in a range of 50% to 90% of patients with liver cirrhosis [6,7]. The grade of OV often correlates with the severity of liver disease. While approximately 85% of individuals with Child-Pugh C have varices, they are present in only 45% of those with Child-Pugh A cirrhosis [6]. OV were identified as one of the factors associated with shorter life expectancy in Tunisians patients with cirrhosis [8].

Liver biopsy remains the accepted standard for histological assessment of liver disease activity and fibrosis, despite such limitations as sampling variability, potential complications of an invasive technique, and subjective scoring. Studies of the natural and treatment-modified histological progression of liver injury and fibrosis have relied on

the adoption of uniform grading and staging criteria [9].

Most of the published studies on the prevalence of OV in cirrhotic patients included those with clinically and/or radiologically documented cirrhosis [6,7]. However, few data are available on the prevalence of OV in CHC patients with bridging fibrosis/cirrhosis [10,11]. To the best of our knowledge, no published study has addressed this issue in Egyptian CHC patients.

Endoscopy is the standard screening test for identifying varices. A less-invasive method would be preferable: Several studies have looked for potential non invasive markers, including low blood platelet counts, dilated portal veins as seen on ultrasonography, low blood albumin levels, the presence of telangiectasias, and increased spleen size [12,13]. Although several independent predictors have been identified, no algorithm has been developed to more narrowly select patients for endoscopic testing [14].

Based on the concept that the development of portal hypertension and hence oesophageal varices is the result of liver fibrosis, non-invasive serum markers of liver fibrosis have been extensively tested not only to predict advanced fibrosis [15-18] but also as predictors of OV in well established liver cirrhosis with promising results [19-23]. However, little is known about the performance of these serum markers in predicting OV in CHC patients with bridging fibrosis (stage 3 fibrosis) or histological cirrhosis (stage 4 fibrosis).

Among these non invasive serum markers, some are routinely available, don't require expensive kits as AST/ALT ratio (AAR) [24], AST to platelet ratio index (APRI) [25], Fib 4 index [26] and BARD score [27].

For financial reasons, the national program for treatment of CHC in Egypt excludes patients with stage 4 fibrosis and patients with stage 3 with endoscopically documented OV from receiving Interferon therapy. Therefore, CHC treatment naïve patients with histological stage 3 fibrosis and stage 4 fibrosis (cirrhosis) are endoscopically screened for OV before starting interferon therapy.

The aim of this prospective study was to determine the frequency of OV in patients with bridging fibrosis (stage 3) and cirrhosis (stage 4) and to assess the clinical utility of simple serum fibrosis markers in predicting OV in these patients as compared to endoscopic screening. From a clinical perspective, an important goal of a noninvasive marker is to identify those with a high probability of OV because this group of patients will not receive Interferon-based therapy. Instead, they will receive prophylactic therapy against variceal bleeding.

Patients and Methods

From January 2008 to January 2009, two hundred CHC consecutive patients were included in the current study. They were presented to the gastrointestinal endoscopy unit of the Department of Tropical Medicine and Gastroenterology, Sohag Faculty of Medicine, Egypt for pretreatment assessment of chronic hepatitis C patients whose histological examination of the liver showed stage 3 or 4 fibrosis according to Desmet et al. [28] staging and grading system. Exclusion criteria were: Co-infection with hepatitis B virus or human immunodeficiency virus (HIV), other causes of liver disease, anticoagulant treatment. None of the patients was receiving β blockers or had history of gastrointestinal bleeding or had undergone endoscopic sclerotherapy or band ligation for OV or transjugular intrahepatic porto-systemic shunt. All patients gave an informed consent and the study was approved by the local ethics committee.

Routine laboratory investigations:

All patients recruited were diagnosed as CHC based on positive anti-HCV antibody test by ELISA done by ARCHITECT kits (provided by Abbott laboratories) and positive HCV RNA by real time PCR for at least 6 months. They had available laboratory investigations including complete blood count using Beckman Coulter, liver function tests (serum bilirubin, proteins, aspartate transaminase, alanine transaminase), fasting serum glucose using autoanalyser Synchronic CX9 System (Bechman coulter, Inc./USA), prothrombin time and concentration.

Histopathological evaluation:

All patients included had liver biopsy report from the Pathology Department, Sohag Faculty of Medicine examined by a single histopathologist who was unaware of the biochemical data of the patient. In general, a sample of 1.5cm in length that was 1.2-2mm in diameter and contains at least 6-8 portal triads was considered adequate. Each was routinely-processed, formalin-fixed, paraffin-embedded according to the usual schedule used in the laboratory. Five micron serial tissue sections were cut, mounted on poly-L-lysine coated slides and dried overnight at room temperature. Sections were de-paraffinized, rehydrated and stained with haematoxylin and eosin (H&E) to assess the degree of liver affection.

All H&E stained sections were then examined to evaluate the necro-inflammatory activity (histological grade) and fibrosis (histological stage) according to Desmet et al. [28] grading and staging system. The histological grade was performed taking into account the necro-inflammation in the portal tracts, periportal and lobular areas: 0=no necro-inflammatory activity, 1=minimal activity, 2=mild activity, 3=moderate activity, 4=severe activity. Fibrosis was evaluated as: 0=no fibrosis, 1=portal fibrosis without septa, 2=few septa, 3=numerous septa delineating nodules without cirrhosis, 4=cirrhosis. Only patients with fibrosis stage 3 and 4 were enrolled in the present study.

Serum fibrosis markers:

For all patients, simple serum fibrosis markers were recorded or calculated from the above mentioned routine laboratory investigations. These include platelet count, AST to ALT ratio (AAR) [24], AST to platelet ratio index (APRI) [25], Fib 4 index [26] and BARD score [27]. Table (1) shows the equations used in calculation of these markers.

Table (1): Equations for calculation of serum fibrosis markers.

Marker	Equation
AST to ALT Ratio (AAR)	AST/ALT
AST to Platelet Ratio Index (APRI)	$[(AST/Upper\ limit\ of\ normal*)/ Platelet\ count\ (10^9/l)] \times 100$
Fib 4 index	$(Age[years] \times AST[IU/L]/(platelet [10^9] \times ALT[IU/L])$
BARD score	Scale 0-4 BMI $\geq 28kg/m^2$=1 point AST/ALT ratio ≥ 0.8=2 points Diabetes mellitus=1 point

* Upper limit of normal for AST in our lab is 37 IU/L.

Since these serum markers were originally generated as a surrogate measurement of liver fibrosis and not for OV, new cut offs for OV were defined according to area under the curve (AUC) analysis.

Endoscopic examination:

All patients underwent an upper GIT endoscopy for detection and grading of oesophageal varices by well trained endoscopists who were unaware with the laboratory and histopathological data of the patients. Varices were classified as absent (grade 0), small (grade 1), medium (grade 2) or large (grade 3) according to Cales et al. [29]. Small oesophageal varices were those that flattened (disappear) during insufflation. Medium varices were not flattened by insufflations and were separated by areas of normal mucosa. Large varices were confluent and not flattened by insufflations.

Statistical methods:

Data entry and analysis were done using SPSS software v17 (Chicago, USA). Continuous values were described by mean and standard deviation. Categorical values were described by counts and proportions. Univariate analysis for determining the association of various clinical and laboratory variables with the stage of liver fibrosis and the presence or absence of OV was performed using Student's *t*-test for continuous variables and χ^2 test for categorical variables. Differences were considered statistically significant if *p*-value was less than 0.05.

To determine the clinical utility and the diagnostic performance of serum fibrosis markers, 2 receiver operating characteristic (ROC) curves were constructed for each of the non-invasive scoring systems that appeared significant in the univariate analysis. The first curve was for prediction of OV of any size, therefore, patients who had varices of any size were considered positive. While those who had no varices were considered negative. The second curve was for prediction of histological cirrhosis (stage 4 fibrosis). Therefore, patients with stage 4 fibrosis (cirrhosis) were considered positive. While, those with stage 3 fibrosis (bridging fibrosis) were considered negative. Performance of the non-invasive markers was expressed as sensitivity, specificity, positive and negative predictive values (PPV and NPV) and test accuracy.

Results

Demographic, clinical, and histological data:

Among the 200 CHC patients included, 190 were males and 10 were females. The mean age of all patients was 40.26±11.26 years. A total of 116 subjects had stage 3 (bridging fibrosis), and 84 subjects had stage 4 fibrosis (cirrhosis). Subjects with stage 4 had significantly lower platelet count (178.79±78.89 vs 242.91 ±77.89; *p*=0.000) and significantly lower synthetic functions (serum albumin) than those with stage 3 (3.52±0.81 vs 3.92±0.92; *p*=0.029). The aspartate aminotransferase (AST), alanine aminotransferase (ALT) levels, APRI and Fib 4 index were all significantly higher in patients with stage 4 than stage 3 fibrosis. No significant difference was noticed between both groups regarding AAR and BARD score.

Endoscopic data:

Endoscopic examination revealed that 100 patients (50%) of the entire group had OV. Forty-two patients (36.2%) of stage 3 had OV (38 small, 2 medium and 2 large). While, Fifty-eight (69%) of patients of stage 4 had OV (42 small, 4 medium

and 12 large). Hence, most (80%) of the detected varices were small.

Laboratory results of patients with and without OV:

When patients were categorized according to the presence or absence of OV at endoscopy, we found that patients with OV have significantly lower serum albumin and platelet count (and significantly higher fasting blood glucose, AST, ALT, and APRI than patients without OV. Diabetes mellitus was significantly more frequent in patients with OV than patients without. On the other hand, no significant difference was found between both groups in AAR, Fib4 index and BARD score (Table 2).

Table (2): Comparison between patients with varices and those with no varices.

	Varices (n=100)	No Varices (n=100)	*p*-value
Age (ys)	41.12±11.45	39.40±11.12	n.s.
BMI (kg/m^2)	26.72±4.34	26.04±4.14	n.s.
Glucose (mg/dl)	107.50±35.89	88.08±18.53	0.001
Diabetes mellitus*	18 (18%)	2 (2%)	0.008
Albumin (gm/dl)	3.57±0.81	3.94±0.95	0.038
ALP (IU/L)	110.38±64.15	94.20±69.27	n.s.
AST (IU/L)	28.94±8.52	17.60±6.82	0.036
ALT (IU/L)	26.34±19.59	17.98±10.54	0.009
T.Bil (mg/dl)	0.85±0.31	0.94±1.15	n.s.
Hb (g/dl)	14.67±2.75	15.39±1.56	n.s.
Plts (x10^9/L)	195.40±88.55	236.56±74.85	0.014
Proth. time (sec)	12.65±2.13	12.61±2.04	n.s.
Proth. Conc (%)	84.26±9.58	83.16±10.26	n.s.
AAR	1.51±0.33	1.07±0.19	n.s.
APRI	0.68±0.19	0.26±0.07	0.035
Fib 4 index	2.07±0.50	1.02±0.37	n.s.
BARD score	2.12±0.12	1.84±0.10	n.s.

Data are expressed as mean ±SD and *p*-value is calculated by student's-*t*- test.

* Data are expressed as numbers & % and *p*-value is calculated by χ^2 test.

BMI	: Body mass index.	T. Bil	: Total bilirubin.
F.S	: Fasting serum.	Hb	: Haemoglobin.
ALP	: Alkaline phosphatase.	Plts	: Platelets.
ALT	: Alanine transaminase.	Proth	: Prothrombin.
AST	: Aspartate tranaminase.	n.s.	: Non significant.

Performance of serum fibrosis markers for prediction of OV/liver cirrhosis:

We performed ROC curve analysis for the 3 serum fibrosis markers that differed significantly in univariate analysis between patients with and without OV (platelet number, APRI and albumin). The AUC, the optimized cut off for each of these markers as resulted by AUC analysis is shown in Table (3). The sensitivity (Sens %), specificity

(Spec %), positive predictive value (PPV) and negative predictive value (NPV) for each marker were calculated twice. For prediction of OV (any grade), the results of the tests were compared with the results of endoscopy taken as the gold standard. For the prediction of histological cirrhosis (stage 4 fibrosis), the results of the tests were compared with the histopathology results as a gold standard. For the prediction of OV, APRI showed the best-although unsatisfactory-performance as indicated by an AUC of 0.64. Meanwhile, this marker showed the best and most satisfactory performance for prediction of liver cirrhosis (AUC=0.801). Fig. (1) shows the receiver operating characteristic curves of APRI, platelet count and serum albumin for prediction of OV.

Table (3): Performance of simple non-invasive serum markers for prediction of any grade of oesophageal varices vs histological cirrhosis.

	AUC	Cut-off	Sens (%)	Spec (%)	PPV (%)	NPV (%)	Accuracy (%)
Plt:							
For OV	0.351	135	86	8	48.3	36.4	47
For LC	0.291	153.5	71.4	6.9	35.7	25	34
APRI:							
For OV	0.642	0.16	70	54	60.3	64.3	62
For LC	0.801	0.16	90.5	65.5	65.5	90.5	76
Albumin:							
For OV	0.382	3.2	70	20	77.8	27.2	45
For LC	0.381	3.2	66.7	19	38	42.9	39

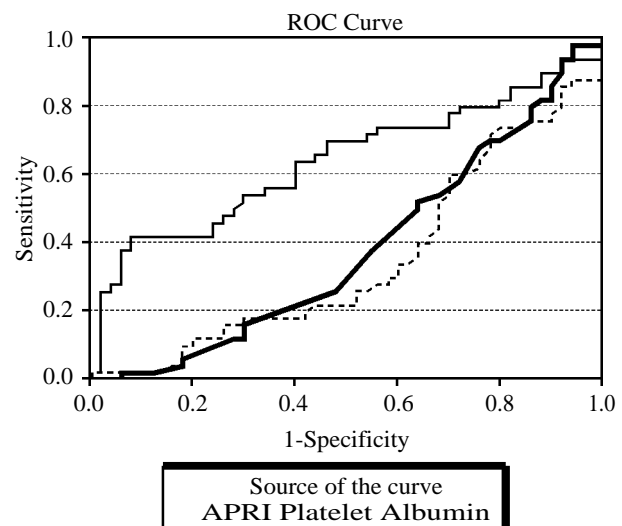|			
AUC : Area under curve.		Plt	: Platelets.
Sens : Sensitivity.		APRI	: AST to platelet ratio index.
Spec : Specificity.		OV	: Oesophageal varices.
PPV : Positive predictive value.		LC	: Liver cirrhosis.
NPV : Negative predictive value.			

Fig. (1): Receiver operating characteristic curves (ROC) for platelet count, serum albumin and APRI for prediction of oesophageal varices in CHC patients.

Discussion

The measure of disease progression represents a key challenge in any of the different stages of chronic liver disease. Indeed, a correct and reliable measure of the stage of the disease has relevant implications for assessing the effectiveness of the current therapeutic regimens and predicting the occurrence of complications [30].

Authoritative guidelines recommend routine upper endoscopy in patients with liver cirrhosis [31]. However, cirrhosis can be overlooked in up to 20% of cases [32], even when a 25-mm long biopsy specimen is available for histologic evaluation. Bedossa et al. [33] and Gentile et al. [34] reported the presence of small OV in 11.1% of patients with histologically diagnosed minimal/mild chronic hepatitis using Ishak staging and grading system [35]. They concluded that varices can be missed in a relatively high proportion of cases if only patients with a diagnosis of cirrhosis undergo upper endoscopy for the screening of gastro-oesophageal varices.

The presence of varices in the eosophagus or stomach (i.e. gastroesophageal varices) in patients with chronic liver disease has important prognostic and therapeutic implications. It was reported that approximately 30% of patients with OV will bleed within the first year after diagnosis [36]. Therefore, patients with moderate to large varices should receive prophylactic beta-blockers or undergo band ligation to reduce the risk of variceal hemorrhage [37]. In addition, patients with varices should avoid aspirin, non-steroidal anti-inflammatory drugs, and other anticoagulants to reduce the risk of gastrointestinal bleeding. Although upper endoscopy is the "gold standard" for diagnosing varices, the cost, risk, and inconvenience associated with this invasive procedure have led many to seek alternative means to predict the presence and severity of varices [38,39].

Our results show that OV are present in 50% of patients with CHC in stage 3 and 4 fibrosis. This frequency is higher than that reported by Sanyal et al. [11] who found OV in 25.5% of CHC patients with bridging fibrosis (Ishak stage 3-4) or compensated cirrhosis (Ishak stage 5-6). Similarly, Gentile et al. [34] found OV in 24% of chronic liver disease patients of viral aetiology. On the other hand, Gill et al. [10] reported OV in 70% of chronic hepatitis of viral aetiology. However, they included patients with a diagnosis of chronic hepatitis with platelet counts of < 140000, ≤I.N.R 1.5,

p-V diameter ≤ 13mm in their study. Therefore, this discrepancy in the frequency of OV in different studies could be explained by the different stages of fibrosis in the recruited patients, different laboratory inclusion criteria, racial differences and whether the diagnosis of liver disease was based on the biopsy or not.

In the current study, 36.2% of patients with bridging fibrosis (stage 3) and 69.1% of patients with stage 4 (cirrhosis) had OV and this is also higher than that reported by Sanyal et al. [11] where it was 16% vs 39% in their study. Cast·era et al. [40] detected OV in 36% of CHC cirrhotic patients. The presence of OV in more than one third of patients with bridging fibrosis in the present study indicates that liver biopsy alone may underestimate the frequency of liver cirrhosis in CHC patients and this confirms previous observations [11,32]. Several studies reported that the accuracy of liver biopsy for the diagnosis of cirrhosis is questioned, in relation to sampling errors and intra-and inter-observer variability that may lead to understaging [33,41,42]. An alternative explanation may be that portal hypertension and consequently OV may develop in the stage of bridging fibrosis before the development of regeneration nodules (true cirrhosis). Therefore, it is possible that a significant portion of patients (36.2% of patients with stage 3 fibrosis in the present study) would elude screening endoscopy if we only relied on liver biopsy for the diagnosis of liver cirrhosis.

In the present study, among patients with varices (n=100) the majority of them (80%) show small varices. Medium varices were found in 6%, and large varices in 14%. This agrees with Sanyal et al. [11] and Gentile et al. [34] where most of the reported OV were small. However, the presence of medium and large varices in 20% of patients with varices (10% of all studied patients) highlights the importance of endoscopic screening in CHC patients with stage 3 and 4 fibrosis as these varices are liable to bleeding so need prophylactic measures. Moreover, it has been reported that Approximately 4-30% of patients with small varices will develop large varices each year and will therefore be at risk of bleeding [36]. In the two years after detection of esophageal varices, the risk of esophageal bleeding ranges from 20-30% and the mortality within one week is approximately 25-30% [29,43].

In the current series, of those without OV, 74% had bridging fibrosis (stage 3 fibrosis) and 26% had liver cirrhosis (stage 4 fibrosis). Conversely,

the majority of subjects with OV had histologically proven liver cirrhosis (58%). This agrees with Sanyal et al. [11]. A follow-up study by Fontana et al. [44] demonstrated the development of De novo varices in 157 of the 598 (26.2%) patients with CHC during a 4 year follow up and most of these new varices were small (76.4%) and only 1% of patients developed variceal hemorrhage.

In the past few years a number of clinical, laboratory, and ultrasonographic variables have been explored as non-invasive alternatives to endoscopy to predict the presence of OV [38]. Among the laboratory variables tested in our study, 5 were significantly associated with OV in univariate analysis (AST, ALT, platelet count, APRI and serum albumin). Another interesting finding in the current series is that diabetes mellitus was significantly more frequent in CHC patients with OV than those without. However, although the association between hepatitis C and diabetes mellitus have been extensively reported [45,46], the impact of diabetes mellitus on portal hypertension and development and/or progression of OV have not been previously investigated. Therefore, longitudinal studies are needed to clarify this point.

Several studies suggested that platelet count may predict the presence of OV in patients with cirrhosis [47-51]. Furthermore, Sanyal et al. [11] found that a low platelet count is predictive of OV in patients with CHC and advanced fibrosis. Our finding of a significantly decreased platelet count in CHC with OV compared to those without OV is in agreement with previous reports. However, ROC curve analysis of our data showed that at a platelet count cut-off of 135, although a good sensitivity (86%) was found, the specificity was very low (8%) and hence a very high false positive rate (92%). Therefore, platelet count is unsuitable for screening these patients for OV.

APRI is a simple index derived from AST and platelet count [25]. It was initially developed for the non invasive prediction of fibrosis and cirrhosis in patients with CHC and showed good performance (AUC of 0.94 for cirrhosis) [52]. When used for predicting OV [40] in cirrhotic patients, APRI didn't perform so impressively (optimum cut off 1.3; AUC=0.62). In their retrospective study, Sebastiani et al. [53], APRI at a cut off 1.4 had an AUC=0.63 for prediction of any grade OV in CHC patients. At the same cut off, Stefanescu et al. [54] reported a poorer performance for prediction of OV (AUC=0.545). Our results are in accordance with these reports as we found an AUC of 0.642 for APRI at a cut off 0.16 and the overall accuracy of

the test was 72% for prediction of OV. The reason of the lower cut off in our study is that non of the included patients had clinical cirrhosis which means that they were in a much earlier stage of the disease than patients in previous studies who were clinically cirrhotic with varying Child grades from A to C.

In CHC patients with advanced fibrosis, Fontana et al. [44] reported that baseline serum albumin and hyaluronic acid levels were significantly associated with an increased risk of developing new varices on follow up which was consistent with prior studies [55] demonstrating that these laboratory parameters correlate with the extent of hepatic fibrosis and disease severity. Gana et al. [56] designed a model including serum albumin together with platelet count and spleen length as a predictor of OV in children with chronic liver disease. Furthermore, lower total serum protein and serum albumin were significantly associated with variceal bleeding in cirrhotic patients [57]. However, our results demonstrated that although serum albumin was significantly lower in CHC patients with OV than those without, ROC curve analysis of our data showed poor performance of the test with an AUC of 0.382 for prediction of OV.

Fib-4 was proved as a good non invasive marker of liver fibrosis in CHC with performances similar to the Fibro Test [15]. It relies on the age, aspartate- and alanine aminotransferase levels and the platelet count, which are routinely measured and available for virtually all subjects with liver disease. It was used also for prediction of OV in cirrhotic patients with a moderate performance [54]. On the contrary, in the present study, no significant difference was noted between patients with and without OV in the Fib-4 level, therefore it can't be used to predict OV in our series with stage 3 and 4 fibrosis. Similarly, BARD score although it was reported to be useful in predicting advanced fibrosis in non alcoholic fatty liver disease [27], we didn't find a significant difference in its level between CHC patients with and without OV.

In conclusion, OV are frequent in Egyptian CHC patients with stage 3 (bridging fibrosis) or stage 4 (cirrhosis) indicating the value of screening endoscopy in these stages. None of the simple tests currently available can accurately predict the presence of OV. Hence, endoscopy remains the best screening test to identify oesophageal varices in CHC with advanced fibrosis. Further studies are needed to clarify the impact of diabetes mellitus on the development and or progression of portal hypertension in CHC patients.

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