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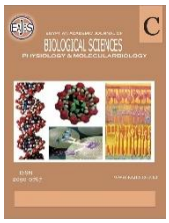

The Use of Circulating Fibronectin and Blood Biomarkers for Non-invasive Assessment of Liver Status Before and After Liver Transplantation

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ABSTRACT

Liver transplantation (LT) remains the treatment of choice for liver cirrhosis (LC) and early-stage hepatocellular carcinoma (HCC). However, HCC recurrence rates remain high, and recurrence is a key predictor of survival post-transplant. This study aimed to investigate the use of circulating Fibronectin (FN) and other blood biomarkers for non-invasive monitoring of liver status pre- and post-transplant. A total of 80 Egyptian patients with chronic hepatitis C (56 males and 24 females, aged 18 - 66 years) and 16 age-matched healthy individuals were included. The patients were 50 with LC and 30 with HCC. Of these patients, 36 patients (22 with LC and 14 with HCC) underwent successful LT and were followed one-year after transplantation to evaluate liver status. Traditional biomarkers and circulating FN levels were assessed and the data were analysed using SPSS. The HCC patients showed significantly higher mean levels of FN in comparison with LC patients and healthy individuals. FN demonstrated an AUROC of 0.878 at a cut-off level of 1.85 mg/L for discriminating HCC from all non-HCC individuals, yielding 92.7% efficiency. One-year after transplantation, a significant decline ($p < 0.05$) was observed in levels of all investigated blood biomarkers and FN compared to pre-transplantation levels. However, patients with pre-HCC exhibited significantly ($p < 0.0001$) higher mean levels of FN (1.865 mg/L) compared to those with pre LC (1.601 mg/L) after transplantation. In conclusion, FN is a highly accurate biomarker for non-invasive monitoring liver status post-transplant. It offers potential for improving LT outcomes and reducing recurrence.

INTRODUCTION

Liver cancer continues to pose a significant global health challenge, with its incidence steadily rising worldwide. Projections suggest that by 2025, approximately 1 million individuals will be diagnosed with liver cancer annually (Yang *et al.,* 2019). Hepatocellular carcinoma (HCC) is the most common form of liver cancer, making up roughly 90% of all liver cancer cases (Llovet *et al.,* 2021). HCC ranks as the fifth most prevalent cancer worldwide and is the second leading cause of cancer-related deaths (Dhanasekaran *et al.,* 2019; Ninio *et al.,* 2019). Annually, over 700,000 new HCC cases are diagnosed, with more than 600,000 people dying from the disease. Males are more frequently affected than females (Sagnelli *et al.,* 2020).

Despite advancements in our understanding of the risk factors associated with HCC, the global incidence and mortality rates of the disease continue to rise (Koshiol *et al.,* 2021). Consequently, developing more efficient therapies remain critical priorities (Kanwal *et al.,* 2017) and early identification of precancerous HCC nodules before they develop into cancerous lesions is crucial for improving the 5-year survival rate and potentially reducing both incidence and mortality (Singal *et al.,* 2020; Gomaa *et al.,* 2008). Liver transplantation and resection are the primary therapeutic approaches for HCC (Assalino *et al.,* 2020). However, the overall prognosis remains poor due to the limited responsiveness of HCC to chemotherapy and radiation, in addition to the high rates of metastasis and recurrence, which constrain available treatment options (Zhang *et al.,* 2020). Biomarkers are measurable indicators of physiological or pathological processes, as well as responses to diagnostic or therapeutic interventions. A number of potential biomarkers for the early diagnosis and prognosis prediction of HCC have been identified (Verde *et al.*, 2020). However, many of these biomarkers are not yet suitable for clinical application due to issues with measurement validity and reproducibility (Llovet *et al.,* 2023). Fibronectin (FN) is a high-molecular-weight glycoprotein found in both the extracellular matrix and circulating plasma (Attallah *et al.,* 2007; Parisi *et al.,* 2017). This large glycoprotein is crucial for mediating cell adhesion, growth, differentiation, and migration. As a key item of the extracellular matrix, FN has been shown to contribute to cancer progression by activating various downstream signalling pathway (Kim *et al.,* 2017). Abnormal FN expression has been associated with HCC progression (Kim *et al.,* 2017). However, despite its potential role in tumorigenesis, FN has not been extensively researched as a biomarker for HCC (Kim *et al.,* 2020). Given this gap, the present study aims to validate the potential of FN as a diagnostic biomarker for HCC. Additionally, the present study explores FN's potential role in monitoring liver status post-transplantation, thereby contributing to improved liver transplant outcomes and a reduced risk of disease recurrence.

MATERIALS AND METHODS

1. Study Patients and Controls:

The present case control study included 80 patients (56 males and 24 females, aged 18 - 66 years) with HCV-related liver diseases and 16 healthy individuals (10 males and 6 females, aged 18 - 66 years) recruited from the Gastrointestinal Surgery Center, Mansoura University, Mansoura, Egypt from March 2022 to June 2023. Liver biopsies were performed for all patients to assess the severity of LC and tumor stages, grades, sizes, and sub-sites of HCC. Standard clinical endoscopic, histologic, and radiographic criteria were used to confirm diagnosis. Fifty patients (34 males and 16 females, aged 18 - 64 years) were diagnosed LC and 30 patients (22 males and 8 females, aged 29 - 66 years) were diagnosed HCC. Our study excluded patients with other cancers or haemolysis, and patients with other viral infections (HBV infection or co-infection with HCV). Of the study patients, 36 patients (22 with LC and 14 with HCC) underwent successful liver transplantation and were monitored for one-year post-transplantation during the period from January 2023 to June 2024. The study was conducted in accordance with the Declaration of Helsinki's ethical standards, and it was approved by the ethics committee of Gastrointestinal Surgery Center, Faculty of Medicine, Mansoura University, and all subjects provided written informed permission.

2. Blood Samples:

Blood samples were collected from healthy individuals and from all patients before and after liver transplantation following standard phlebotomy procedures. A total of 10 mL blood were drawn from each participant and subdivided into two portions. One portion of was treated with EDTA and tested for routine haematology. The second portion was putted into additive-free tube and

left to clot for 10 minutes at room temperature. Then, the serum was separated by centrifugation at 1000 ×g for 3 minutes at 4 °C. The serum was aliquot, labelled and stored at −80 °C until analysis.

3. Biomarkers and Traditional Laboratory Tests:

Fresh sera will be processed for the biochemical analysis of Albumin, Total protein, Total bilirubin, Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), γ-Glutamyl Transferase (γGT) and Creatinine (Creat.) according to the Manufacture instructions. A complete blood count, including Platelet (Plt) count, was performed on blood samples treated with EDTA using the KX-21 Sysmex automated haematology analyser (Sysmex Corporation, Kobe, Japan). For prothrombin-INR (International Normalized Ratio), a separate portion of the blood was treated with a citrate solution. Serum levels of AFP tumor marker were assessed using sandwich ELISA.

4. Measurement of FN Using Sandwich ELISA:

The levels of FN biomarker were assessed in serum using a commercially available ELISA kit (Cat no. ELK1020, ELK Biotechnology, Denver, CO 80202, USA), based on the manufacturer's guidelines by using a microplate reader (Biochrom EZ Read 400 Microplate Reader, Cambridge, UK) set to 450 nm. Standard curves representing the relationship between optical density (OD) and the standard concentrations were established. By comparing the sample's OD to the standard curve, the concentrations of circulating FN in mg/L were determined in the investigated samples.

5. Statistical Analysis:

A two-sided $P < 0.05$ was used to determine statistical significance for all statistical analyses, which were performed using the statistical software program SPSS 25.0 for Microsoft Windows. The median (range) was used to express most of numerical data. The Mann-Whitney test and Kruskal Wallis test were utilized to examine the marker levels and compare them between independent groups. We computed the Area Under Curve (AUC) for the Receiver operating characteristic (ROC) curve. The AUC varies from 0.5 (indicating a non-informative marker) to 1 (indicating a perfect marker). It represents the likelihood that a randomly chosen case will have a higher marker value than a randomly chosen control. The best cut-off values for the examined markers with sensitivity and specificity were found by plotting ROC curves.

RESULTS

1. Levels of Investigated Blood Biomarkers for Discriminating LC and HCC Patients:

1.1. Biochemical and Haematological Markers:

The distribution of evaluated biochemical markers AST, ALP, BIL, ALB, Creatinine differed significantly. There are statistically highly significant ($p < 0.0001$) differences in patients with HCC compared with LC patients and healthy individuals. There is no significant ($P > 0.05$) difference in AST/ALT ratio in patients with HCC compared with LC patients and healthy individuals (Table 1). The value of Plt count was significantly ($p < 0.05$) lower in HCC compared to LC patients and healthy individuals. Also, the value of INR slightly decreased in HCC compared to LC patients (Table 1). HCC patients showed high levels of AFP (4 ng/mL; 1.1 to 300) in comparison with LC patients (3.1 ng/mL; 0.7 to 72) and healthy controls (1 ng/mL; 0.7 to 1.2) (Table 1 and Fig. 1a).

1.2. Fibronectin:

The serum levels of FN in HCC patients (1.9 mg/L, 0.71 to 2.07) were significantly higher ($p < 0.05$) than that of LC patients (1.69 mg/L, 1.16 to 1.84) and of healthy controls (1.02 mg/L, 0.74 to 1.81) as shown in Table 1 and Figure 1b. Moreover, levels of circulating FN increase with the increase in tumor size and grade (Fig. 2a, b).

2. Performance Characteristics of AFP and Fibronectin for discriminating HCC Patients:

The AUROC (area under ROC curve) of all laboratory biomarkers for discriminating HCC patients from LC

patients and healthy controls were determined. Traditional tumor marker AFP showed AUROC of 0.719 at cut-off of 0.8 ng/ml for discriminating HCC from non-HCC individuals. AFP also demonstrated an AUROC of 0.994 for distinguishing HCC from healthy controls, an AUROC of 0.631 for differentiating HCC from LC patients, and also showed AUROC of 0.921 for differentiating LC patients from healthy controls **(**Table 2 and Fig. 3a-d). However, FN showed the highest AUROC of 0.878 at cut-off of 1.85 mg/L for discriminating HCC from non-HCC individuals, yielding a sensitivity of ~ 77%, specificity of 100%, and efficiency of ~ 93%. Moreover, FN demonstrated strong diagnostic performance, with an AUROC of 0.946 for distinguishing HCC from healthy controls. FN also showed an AUROC of 0.856 for differentiating HCC from LC patients, and also showed AUROC of 0.883 for differentiating LC patients from healthy controls at a cut-off of 1.14 mg/L (Table 2; Fig. 4a-d).

3. Assessment of Traditional Blood Biomarkers and fibronectin before and after Liver Transplantation:

One year after liver transplantation, a dramatic change in levels of investigated markers were reported post-transplantation compared to pre-transplantation levels,Table 3.

3.1. Traditional Biochemical, Haematological and Tumor Markers:

A significant reduction in the levels of AST, ALT, AST/ALT ratio, and Total Bilirubin was observed post-transplantation compared to pre-transplantation levels. Conversely, an increase in GGT, Albumin, Total Protein, and Creatinine levels was noted. However, no significant change was observed in ALP levels following transplantation. Platelet (PLT) counts approximately doubled post-transplantation, while INR levels demonstrated a significant decrease. AFP mean levels decreased from 3.65 ng/mL pre-transplantation to 2.4 ng/mL post-transplantation. In contrast, AFP levels showed a marginal increase from 2.4 ng/mL to 2.41 ng/mL post-transplantation in HCC patients compared to those with liver cirrhosis ($p > 0.0001$), Table 3.

3.2. Fibronectin:

Fibronectin mean levels exhibited a significant decrease one-year post-transplantation compared to that pre-transplantation ($p < 0.05$), Table 3. However, 4 out of 14 patients diagnosed with HCC pre-transplantation and 5 out of 22 diagnosed with LC pre-transplantation showing levels above the cut-off value of FN (1.85 mg/L for HCC and 1.14 mg/L for LC); respectively.

Table 1. Demographic and laboratory data of study patients with liver cirrhosis and HCC in comparison with healthy individuals.

Characteristics[#]		Healthy Individuals (N = 16)	Patients with LC (N = 50)	Patients with HCC (N = 30)	P value*
Demographic data:					
Age (yr)		28.5 (20-51)	46.5 (18-64)	58 (29-66)	P1 > 0.05; P < 0.05; P3 < 0.0001
Sex	**Male**	12 (75%)	34 (68%)	22 (73.3%)	P1 > 0.05; P < 0.05; P3 < 0.0001
	Female	4 (25%)	16 (32%)	8 (26.7%)	
Biochemical markers:					
AST (U/ml)		20.5 (20 to 25)	35 (20 – 303)	34.5 (21 – 127)	P < 0.0001
ALT (U/ml)		27 (16 to 53)	40 (15 – 171)	37 (14 – 158)	P = 0.044
ALT/AST		0.76 (0.38 to 1.4)	0.88 (0.29 - 8.2)	1 (0.4 – 1.5)	P = 0.390
GGT (U/L)		22.5 (20 to 38)	46 (5 – 582)	45.5 (12 – 486)	P = 0.005
Alk. Phos. (U/L)		5 (3 to 5)	6.5 (5 – 45)	6.5 (5 – 42)	P < 0.0001
T. Prot. (mg/dL)		7.1 (6.8 to 8.2)	5.76 (3.2 - 7.52)	5.6 (3.5 - 8.2)	P < 0.0001
Albumin (g/dL)		4.45 (4 to 4.8)	3.4 (2 - 4.7)	3.5 (2.2 - 4.9)	P < 0.0001
T. Bil. (mg/dL)		0.5 (0.5 to 0.9)	1.6 (0.5 - 39.6)	1.95 (.5 - 9.1)	P < 0.0001
Creatinine (mg/dL)		0.78 (0.6 to 1.0)	0.92 (0.7 - 1.4)	1.1 (0.7 - 1.5)	P < 0.0001
Hematological markers:					
Plt Count (10^9/L)		218.3 (163 to 289.1	92.73 (22 – 716)	74.2 (29 – 290)	**P < 0.0001**
INR		1.0 (1.0 to 1.0)	1.3 (1 - 3.7)	1.25 (1 - 2.3)	**P < 0.0001**
Tumor marker:					
AFP (ng/mL)		1.0 (0.7 to 1.2)	3.1 (0.7 – 72)	4 (1.1 - 300)	**P < 0.0001**
Target marker:					
FN (ng/mL)		1.02 (0.74 - 1.81)	1.7 (1.2 - 1.85)	1.9 (0.7 - 2.1)	**P < 0.0001**

Abbreviations: GGT, gama glutamyl transferase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALB, Albumin; T.BIL, total bilirubin; T.PROT, total protein; Alk. Ph, Alkaline phosphatase; WBCs, white blood cells; Plt; platelets, INR, international normalized ratio; AFP, alpha fetoprotein.

[#]Biochemical markers, Hematological markers, Tumor markers are represented as Median and interquartile range (25-75%); Kruskal Wallis tests were used for data analysis. Sex is represented as frequency and percent.

* P > 0.05 is considered not significant; P < 0.05 considered significant. P1 represents difference between healthy individuals and LC patients; P2 represents the difference between healthy individuals and HCC patients; P3 represents difference between LC and HCC.

Table 2. The diagnostic potential of traditional AFP tumor marker and FN for distinguishing study groups.

Markers	Comparison Groups	AURO	*P* Value	Cut-off	Sensitivity %	Specificity %
AFP	HCC & (Healthy + LC)	0.719	P = 0.001	0.8 ng/ml	60	70
	HCC & Healthy	0.994	P < 0.0001	1.3 ng/ml	96.7	100
	HCC & LC	0.631	P = 0.051	2.55 ng/ml	80	44
	LC & Healthy	0.921	P < 0.0001	1.3 ng/ml	82	100
FN	HCC & (Healthy + LC)	0.878	P = 0.001	1.85 mg/L	76.7	98.5
	HCC & Healthy	0.946	P < 0.0001	1.2 mg/L	100	100
	HCC & LC	0.856	P < 0.0001	1.85 mg/L	76.7	98
	LC & Healthy	0.883	P < 0.0001	1.14 mg/L	100	87.5

Abbraviations: LC, liver cirrhosis; HCC; Hepatocellular carcinoma.

Table 3. Demographic data of 36 patients underwent liver transplantation before LT and after one-year post-transplantation.

Characteristics[#]		Patients with Liver Cirrhosis		P value*	Patients with HCC		P value*
		Before LT (n = 50)	*After LT (n = 22)*		*Before LT (n = 30)*	*After LT (n = 14)*	
Demographic data:							
Age (yr)		**46.5 (18-64)**	**44.5 (18-62)**	**0.416**	**58 (29-66)**	**59 (48-66)**	**0.121**
Sex	**Male**	**34 (68%)**	**14 (63.6%)**	**…..**	**22 (73.3%)**	**13 (92.9%)**	**…**
	Female	**16 (32%)**	**8 (36.4%)**		**8 (26.7%)**	**1 (7.1%)**	
Biochemical markers:							
AST (U/ml)		**35 (20 – 303)**	**35 (20 - 119)**	**0.927**	**34.5 (21 – 127)**	**28 (21 to 89)**	**0.465**
ALT (U/ml)		**40 (15 – 171)**	**43.5 (15 - 171)**	**0.760**	**37 (14 – 158)**	**28 (14 - 158)**	**0.262**
ALT/AST		**0.88 (0.29 - 8.2)**	**0.88 (0.3 - 2.5)**	**0.696**	**1 (0.4 – 1.5)**	**1.12 (0.56 -1.5)**	**0.043**
GGT (U/L)		**46 (5 – 582)**	**103.5 (18 - 582)**	**0.028**	**45.5 (12 – 486)**	**44 (20 - 486)**	**0.496**
Alk. Phos. (U/L)		**6.5 (5 – 45)**	**9 (5 - 35)**	**0.177**	**6.5 (5 – 42)**	**5 (5 - 42)**	**0.049**
T. Prot. (mg/dL)		**5.76 (3.2 - 7.5)**	**5.92 (4.6 - 7.5)**	**0.337**	**5.6 (3.5 - 8.2)**	**6.58 (3.7 - 8.4)**	**0.078**
Albumin (g/dL)		**3.4 (2 - 4.7)**	**3.65 (2.6 - 4.7)**	**0.107**	**3.5 (2.2 - 4.9)**	**3.95 (2.2 - 4.9)**	**0.178**
T. Bil. (mg/dL)		**1.6 (0.5 - 39.6)**	**1.6 (0.5 - 39.6)**	**0.946**	**1.95 (.5 - 9.1)**	**1.3 (0.5 - 6.5)**	**0.364**
Creatinine (mg/dL)		**0.92 (0.7 - 1.4)**	**0.92 (0.7 - 1.4)**	**0.879**	**1.1 (0.7 - 1.5)**	**1.1 (0.7 - 1.5)**	**0.970**
Hematological markers:							
Plt Count (10^9/L)		**92.7 (22 – 716)**	**129 (23 - 716)**	**0.043**	**74.2 (29 – 290)**	**180 (69 - 290)**	**0.006**
INR		**1.3 (1 - 3.7)**	**1.15 (1 - 3.7)**	**0.050**	**1.25 (1 - 2.3)**	**1.0 (1.0 - 2.3)**	**0.045**
Tumor marker:							
AFP (ng/mL)		**3.1 (0.7 – 72)**	**2.4 (0.7 - 9.3)**	**0.149**	**4 (1.1 - 300)**	**2.41 (1.1 – 3.2)**	**0.001**
Target marker:							
FN (ng/mL)		**1.7 (1.2 - 1.85)**	**1.6 (1.16 - 1.77)**	**0.029**	**1.9 (0.7 - 2.1)**	**1.865 (1.398 - 1.868)**	**0.018**

Abbreviations: GGT, γ- glutamyl transferase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALB, Albumin; T.BIL, total bilirubin; T.PROT, total protein; Alk. Ph, Alkaline phosphatase; WBCs, white blood cells; Plt; platelets, INR, international normalized ratio; AFP, alpha fetoprotein.

[#]Data are represented as Median and Interquartile Range (IQR 25-75%); Kruskal Wallis tests were used for data analysis. Sex is represented as frequency and percent.

* $P > 0.05$ is considered not significant and p value < 0.05 is considered significant. The difference between groups was calculated using Mann-Whitney Test.

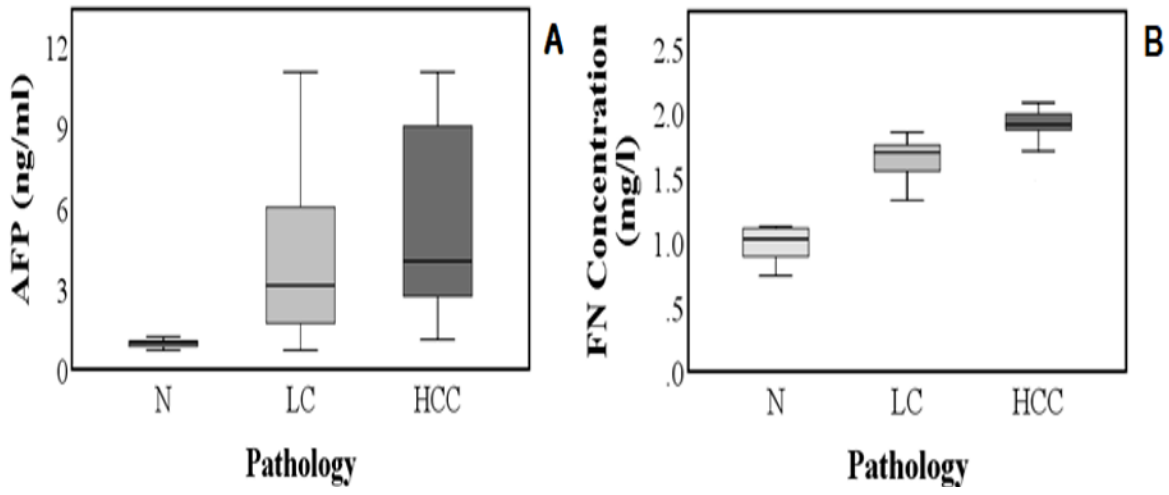

Fig. 1. The concentration of AFP and FN in each group of individuals included in the study.

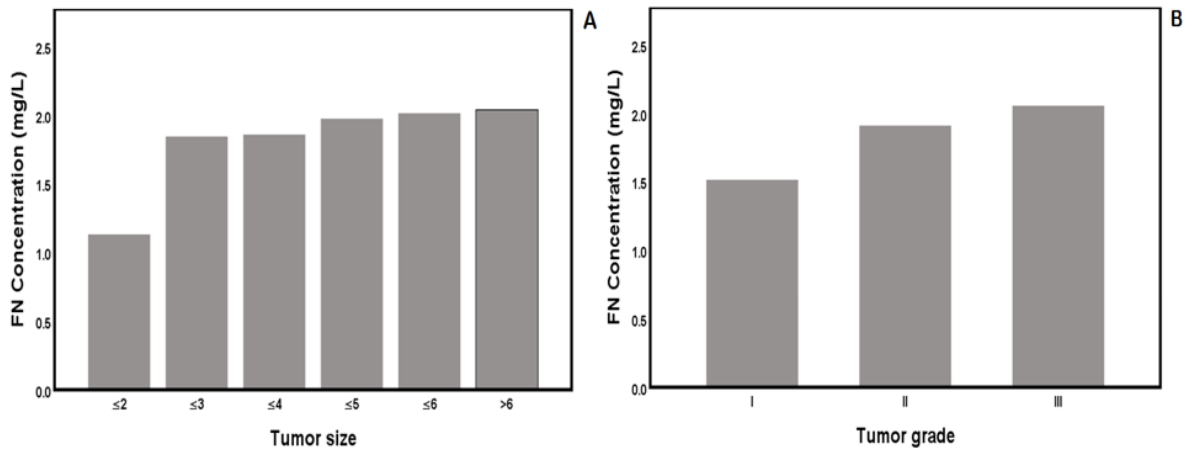

Fig. 2. FN vs. tumor size and tumor grade in individuals included in the study.

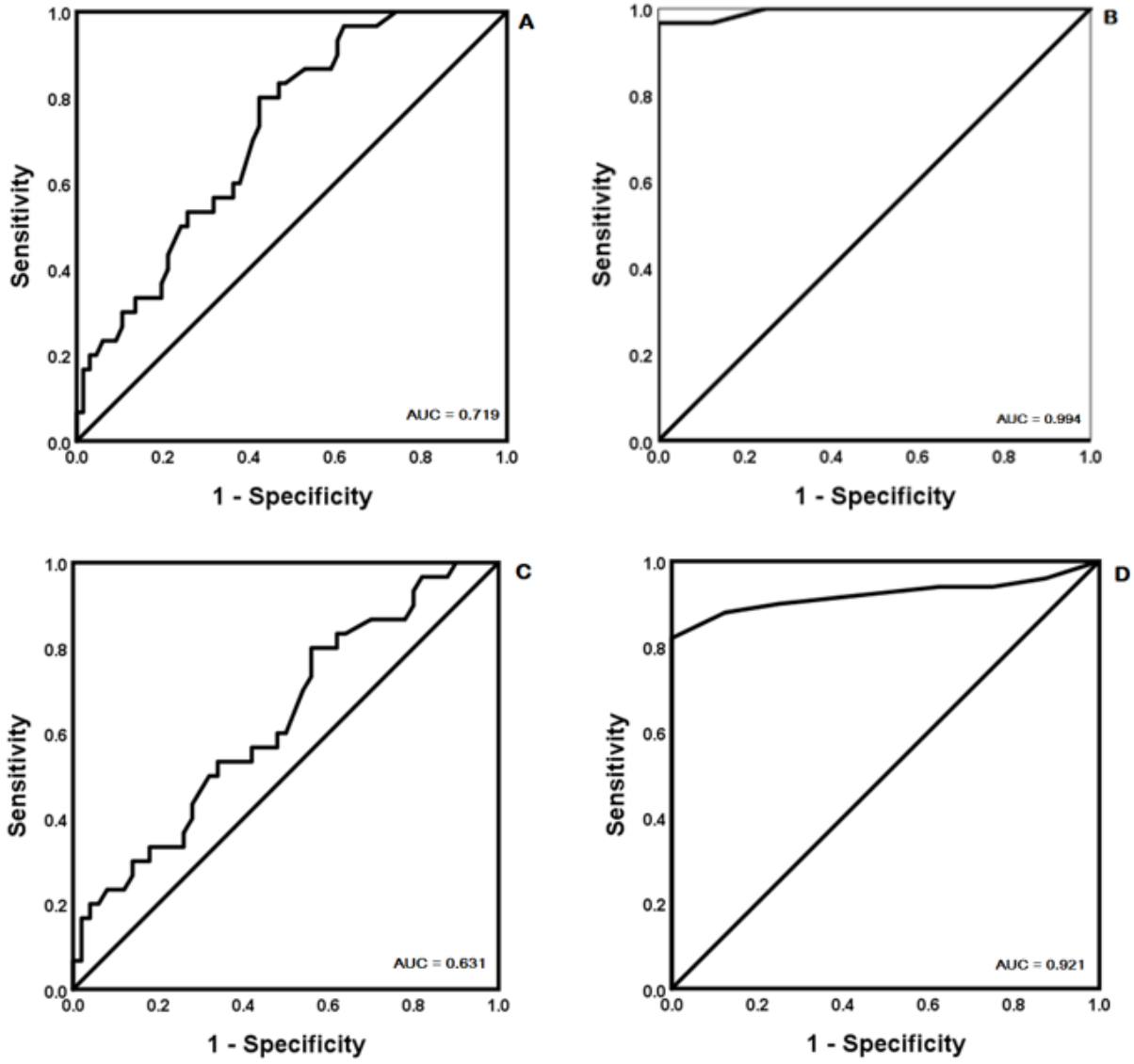

Fig.3. The ROC curves and AUC for AFP to differentiate between: **A.** HCC patients and Nonmalignant individuals (LC patients + Healthy controls); $P < 0.0001$. **B.** HCC patients and healthy individuals ($P < 0.0001$), **C.** HCC patients and LC patients ($P < 0.0001$) & **D.** LC patients and healthy individuals (P value < 0.0001).

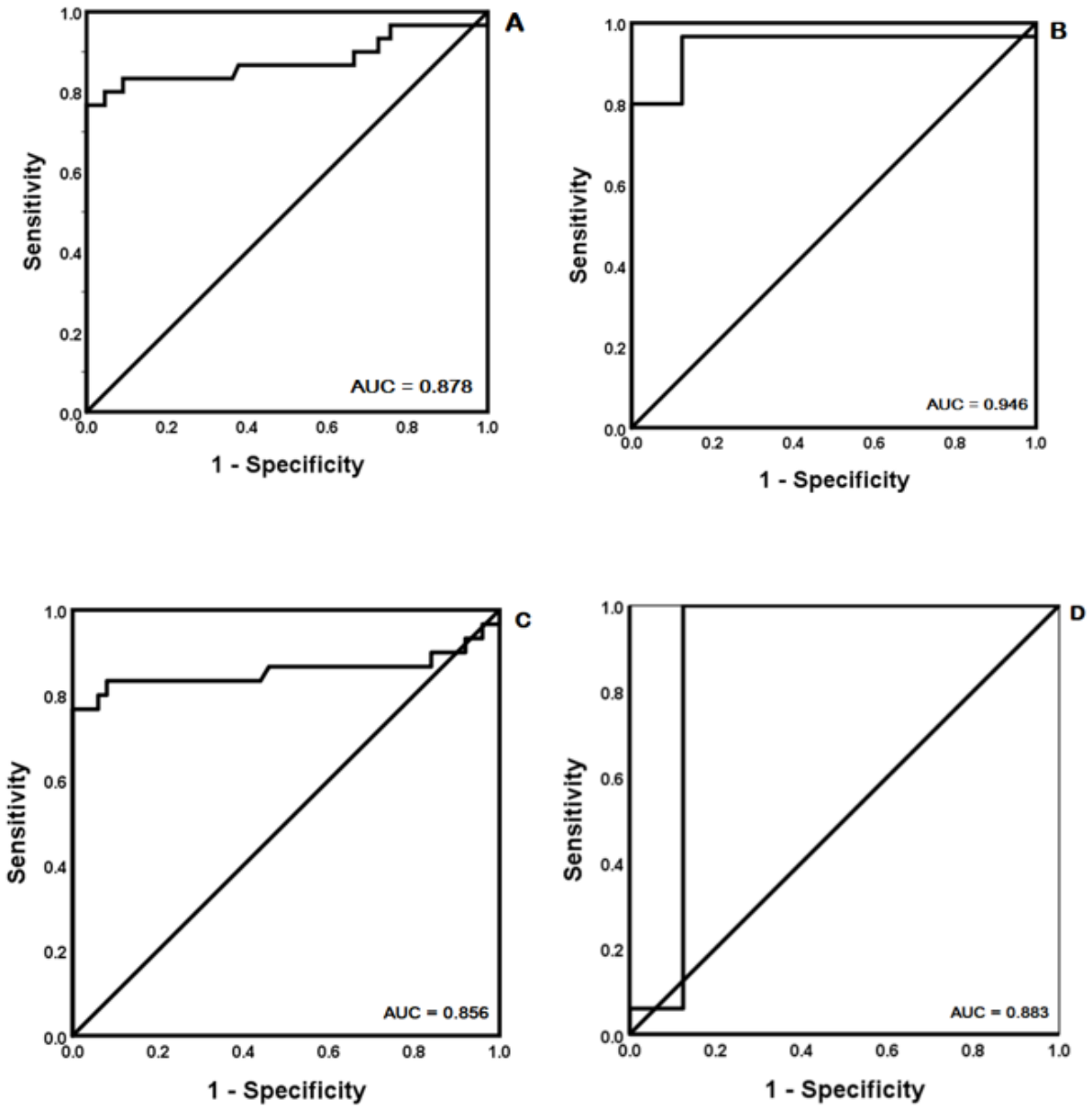

Fig.4. The ROC curves and AUC for FN to differentiate between: **A.** HCC patients and Non-malignant individuals (LC patients + Healthy controls); P < 0.0001. **B.** HCC patients and healthy individuals (P < 0.0001), **C.** HCC patients and LC patients (P < 0.0001) & **D.** LC patients and healthy individuals (P value < 0.0001)**.**

DISCUSSION

Liver cancer, particularly HCC, poses a significant health challenge, being the most prevalent type of primary liver cancer. HCC commonly develops as a result of chronic liver damage, such as from viral infections. HCV is a leading cause of HCC both in Egypt and globally. Studies indicate that 30% of chronic hepatitis C (CHC) patients progress to cirrhosis, and 90% of HCC cases arise in a cirrhotic environment (Khatun *et al.,* 2021). The difficulty in diagnosing HCC early stems from its subtle morphological characteristics, which reduce the chances for timely detection. This delay in diagnosis often leads to patients missing out on appropriate treatment when it is most beneficial. Less than 50% of newly diagnosed HCC patients are able to access suitable treatment options (Chang *et al.,* 2022). Despite the availability of various diagnostic techniques for HCC, approximately 70–80%

of cases are diagnosed late, primarily due to inadequate early detection and lack of regular monitoring of high-risk individuals (de Martel *et al.,* 2018). In recent years, serum biomarkers have become widely utilized for tumor detection, with AFP being the most common marker for diagnosing HCC. However, AFP's diagnostic accuracy is significantly influenced by the chosen cutoff values, with higher cutoff values resulting in decreased sensitivity but improved specificity (Stefaniuk *et al.,* 2010). The European Association for the Study of the Liver (EASL) has excluded AFP from its guidelines for risk-based management of HCC in cirrhotic patients, due to its limited sensitivity and specificity (Sanad *et al.,* 2024). These limitations highlight the need for more reliable biomarkers to aid in the early detection of HCC. Fibronectin (FN), an extracellular matrix protein, has shown promise in contributing to carcinogenesis, including the progression of HCC (Torbenson *et al.,* 2002). In this study, we aimed to investigate the use of circulating FN and other blood biomarkers for non-invasive monitoring of liver status pre- and post-transplant, aiming to improve LT outcomes and reduce recurrence. Our findings indicate that FN levels are significantly higher in HCC patients compared to cirrhotic patients and healthy individuals. Specifically, HCC patients had FN levels of 1.9 mg/L (ranging from 0.71 to 2.07), cirrhotic patients had levels of 1.69 mg/L (ranging from 1.16 to 1.84), and healthy individuals had levels of 1.02 mg/L (ranging from 0.74 to 1.81), with a p-value of 0.0001. Moreover, elevated FN levels were associated with tumor progression, as patients with tumors larger than 3 cm exhibited higher FN levels compared to those with tumors smaller than 3 cm. To evaluate FN's ability to differentiate between cirrhosis and early-stage HCC, we performed a receiver operating characteristic (ROC) curve analysis. The area under the curve (AUC) for FN levels was 0.878, demonstrating strong diagnostic performance for identifying early-stage HCC, specifically in patients with tumors smaller than 3 cm, single nodules, no vascular invasion, and a Child-Pugh score of class A. FN showed a sensitivity of 76.7%, a specificity of 100%, and an efficiency of 92.7%. These results suggest that FN is a highly effective biomarker for early-stage HCC, surpassing other diagnostic techniques in terms of sensitivity. For comparison, previous studies have reported lower sensitivities for AFP and ultrasound in detecting early-stage HCC, with AFP sensitivity about 60 % (Table 2) and ultrasound sensitivity at 47% (Tzartzeva *et al.,* 2018). Additionally, AFP's sensitivity for detecting small HCC lesions is lower, at 54% for single small lesions (Farinati *et al.,* 2006), whereas FN demonstrated a sensitivity of ~ 77 % for identifying single HCC nodules in our study. Other biomarkers, such as AFP-L3 and des-gamma-carboxyprothrombin (DCP), also show relatively low sensitivity in detecting early HCC, with AFP-L3 identifying small HCC lesions with only 61% sensitivity (Lim *et al.,* 2016), and DCP showing a range of 34%–62% sensitivity (Parikh *et al.,* 2020). In a study involving 416 patients, the sensitivity of DCP and AFP-L3 combined was ~ 70% (Huang *et al.,* 2023). In contrast, FN alone exhibited a superior sensitivity of 77% for detecting early HCC. These encourage results enhanced our efforts to investigate the use of FN marker to non-invasive follow up of liver status after liver transplantation. Post-transplant, the recurrence of HCC occurs most frequently (~ 60%) in the first 2 years (Clavien *et al.,* 2012), and this early recurrence is predictive of worse prognosis, often with the increased disease burden and extrahepatic metastases (Ho *et al.,* 2020). No consensus or best practice guidelines for post-transplantation cancer surveillance and recurrence management for HCC currently exist. Studies with adequate population sizes and high-level evidence are lacking (Rajendran *et al.,* 2022). One year following liver transplantation, a significant decrease in FN concentrations was observed post-transplantation compared to pre-transplantation levels. However, 4 patients diagnosed with HCC pre-transplantation showing levels above the cut-off value of FN

(1.85 mg/L for HCC). These indicate that patients who may experience HCC recurrence after LT exhibited higher FN concentrations compared to those who underwent successful LT without recurrence. The study findings underscore the promising role of FN as a diagnostic biomarker for both early stages of HCC and as a tool for monitoring liver status post-transplant. It offers potential for improving LT outcomes and reducing recurrence.

Conclusions

A primary objective in medical research is the identification of an optimal biomarker that can accurately diagnose early-stage HCC in highly vulnerable patients, such as those with liver cirrhosis, thereby minimizing the associated health and social burdens. In this context, the present study aimed to validate the use of circulating FN and other blood biomarkers for non-invasive monitoring of liver status pre- and post-transplant to improve LT outcomes and reduce recurrence. FN exhibited remarkable efficiency for diagnosing HCC from LC. Moreover, FN serves as a potential biomarker for monitoring liver status after LT. However, the improved prediction values of FN should be validated by a larger-scale study.

DECLERATIONS

Ethical Approval: The study was approved by the Research Ethics Committee of the Gastrointestinal Surgery Center, Faculty of Medicine, Mansoura University, Mansoura, Egypt.

Authors contributions: HI and AMA. contributed to conception and design of the study. HAR, and AMA conducted laboratory investigations, collection of data and statistical analysis. MA: clinical investigations. All the authors made a significant contribution to the research and interpretation of the results. HI and HAR drafted the first version of the manuscript. All the authors have read, thoroughly reviewed and approved the final version of the manuscript for publication.

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Availability of Data and Materials: All data supporting the described findings of the study can be obtained from the corresponding authors (HI) upon request.

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