RESEARCH ARTICLE

Potential Diagnostic Role of Serum Fibroblast Growth Factor-19 in Hepatocellular Carcinoma

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Abstract

  Introduction: A highly accurate diagnostic method is crucial to reduce mortality and increase hepatocellular carcinoma (HCC) survival. Current biomarkers have limited accuracy, and novel ones are needed. Fibroblast growth factor-19 (FGF-19) is overexpressed in HCC. This study aimed to assess FGF-19 as a potential novel diagnostic biomarker for HCC. **Methods:** This case-control study involved 114 individuals divided into three equal groups: HCC (n=38), Cirrhosis (n=38), and Control (n=38). HCC biomarkers included alpha-fetoprotein (AFP), Des-γ-carboxy prothrombin (DCP), and FGF-19. **Results:** The three markers, FGF-19, DCP, and AFP, were significantly different between the three groups, except that DCP was comparable between HCC and Cirrhosis groups (p=1.000). All individuals in the control group had FGF-19 levels below the minimum level in the HCC group. Thus, FGF-19 had 100% sensitivity and specificity in differentiating HCC from healthy controls. FGF-19 can discriminate between HCC and Cirrhosis groups at a 140.8 pg/mL cutoff with sensitivity and specificity of 81.8% and 87.9%, respectively. The sensitivity of FGF-19 was higher than AFP, trending toward statistical significance (p=0.095). Combining FGF-19 with AFP, DCP, or both improved sensitivity but decreased specificity. **Conclusion:** FGF-19 is a possible noninvasive serum biomarker for HCC. Its combination with AFP or DCP improves the sensitivity for detecting HCC.

Keywords: Alpha-Fetoprotein- carcinoma- liver neoplasms- liver cirrhosis- biomarkers- fibroblast growth factors

Introduction

In 2020, hepatocellular carcinoma (HCC) was the sixth most prevalent cancer (4.7%) and the third leading cause of cancer-related death (8.3%) [1]. In Egypt, it is the leading cause of cancer-related mortality and morbidity [2], with the highest age-standardized incidence rate (ASIR) (34,1 per 100,000) in Africa and the Middle East [3]. HCV is the leading cause of head and neck cancer in Egypt, followed by HBV [4].

HCC is a highly malignant disease with aggressive invasion, rapid progression, and poor prognosis [5]. Symptoms are absent in the early stages, but the patients reach the middle and late stages at diagnosis [6]. Developing a high-accuracy diagnostic method is crucial to reduce disease mortality and increase patient survival time [5].

Abdominal US has an acceptable sensitivity of 84% for detecting HCC at any stage, but its sensitivity for early-stage disease is lower at 47% [7]. Its effectiveness is affected by operator expertise and patient-level factors like obesity and liver disease severity [8]. In clinical practice, utilization of alternative imaging modalities, such as CT and MRI, is increasing, but scant information on cross-sectional imaging for HCC surveillance [9].

Therefore, serum biomarkers have gained interest in early HCC detection. Many serum biomarkers are available, but they have low sensitivity and varying specificity, even when evaluated in combination with other serum biomarkers [10]. Serum alpha-fetoprotein (AFP) is the most commonly used biomarker. However, its specificity is weakened by its elevation in other benign and malignant conditions [10]. Thus far, there is ongoing debate surrounding the optimal threshold of AFP for diagnosing HCC [11]. Current guidelines recommend ultrasonography (US) with or without serum alpha-fetoprotein (AFP) for the early detection of HCC in high-risk populations [12]. A meta-analysis found that concomitant use of ultrasound and AFP improved early HCC detection, with 63% and 45% sensitivities, respectively [7]. Another biomarker with a high positive rate in the serum of HCC patients is des-γ-carboxy prothrombin (DCP). It is an abnormal form of prothrombin, also found in the serum of patients with vitamin K deficiency [13].

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Several other tumor biomarkers, including insulin growth factor-1 (IGF-1), Serum dickkopf-1 (DKK1), and Golgi protein 73 (Gp-73), have been suggested. Lower levels of IGF-1 in cirrhotic patients with HCV were found to be linked to the development of HCC [14]. Serum dickkopf-1 (DKK1) was suggested to be a new biomarker for HCC, showing excellent diagnostic accuracy even in the early stages and in patients with normal AFP levels [15]. In a case-control study, the Gp-73 was evaluated in HCC patients. However, its early detection had a high false-negative rate of 38%, which hindered its acceptance as a new diagnostic tool for HCC [16]. In recent years, there has been a growing interest in using genomic profiling and proteomics for the diagnosis of HCC [17]. There is a strong correlation between plasma micro-RNA and the development of cancer and the spread of tumors [18].

Among numerous well-known and newly discovered oncogenes associated with HCC, FGF-19 is overexpressed in HCC [19]. The fibroblast growth factors (FGFs) family consists of a sizable group of growth factors found in various multicellular organisms [20]. FGFR1, FGFR2, FGFR3, and FGFR4 are the four transmembrane fibroblast growth factor receptors (FGFRs) that transmit signals from FGFs. Numerous biological processes, including embryonic development, cell proliferation, differentiation, and tissue repair, are regulated by FGFs-FGFRs. The dysregulation of FGF-FGFR has also been extensively reported in numerous diseases, disorders, and malignancies [21]. Notably, aberrant FGF19/FGFR4 expression contributes to the progression of HCC [22].

This study aimed to assess FGF 19 as a potential novel diagnostic biomarker for HCC alone or combined with other biomarkers.

Materials and Methods

This cross-sectional study involved 114 individuals divided into three equal groups: HCC (n=38), Cirrhosis (n=38), and Control (n=38). They were recruited from Cairo University Hospitals from October 2021 to December 2022. The study was approved by the Scientific Ethical Committee of the Faculty of Medicine, Cairo University. Informed written consent was signed by all the patients before enrollment, with an explanation of all the study procedures.

The diagnosis of HCC was based on contrast-enhanced imaging findings using triphasic CT abdomen and pelvis or Dynamic MRI with or without elevated AFP as per the diagnostic criteria of the AASLD [23]. Cirrhosis was diagnosed based on clinical manifestations, laboratory data, and ultrasound findings. Control subjects were selected from patients presenting to the internal medicine clinic complaining of mild gastrointestinal symptoms, nausea, vomiting, or diarrhea. Inclusion criteria for all subjects were age between 18 and 80 of both sexes. HCC patients who received any therapeutic intervention like percutaneous ablation, transarterial embolization, or resection were excluded from the study. All participants were subjected to full detailed medical history and clinical examination. Laboratory investigations included routine

CBC, liver biochemical profile, and viral markers: HCV Ab and HBsAg. The serum specimens were obtained after at least 8-10 hours of fasting and stored at -80°C before laboratory testing. HCC biomarkers included AFP (Chemiluminescence enzyme immune assay), DCP, and FGF-19 (Sandwich ELIZA technique). An ultrasound examination documented signs of liver cirrhosis, portal hypertension, or HCC [focal lesion(s), portal vein thrombosis, and lymph node enlargement]. Triphasic CT or Dynamic MRI was done to confirm the diagnosis of HCC based on contrast uptake and washout. Child-Turcotte-Pugh (CTP) score was calculated for cirrhosis and HCC groups [24]. Serum AFP levels were determined using a chemiluminescent immunoassay analyzer (UniCel DxI 800, Beckman Coulter, Brea, CA, USA). This AFP ELISA kit is an enzyme-linked immunosorbent assay (ELISA) for in vitro quantitative determination of α-fetoprotein (AFP) concentrations in the range of 2-400ng/mL in human serum or plasma samples (Figures 1, 2).

An enzyme-linked immunosorbent assay (ELISA) kit (human AXL DuoSet ELISA, R&D Systems, Minneapolis, MN, USA; human abnormal prothrombin ELISA, Hotgen, Beijing, China) was used to detect DCP levels. The FGF-19 assay is a quantitative sandwich ELISA. The immunoplate was pre-coated with a rabbit polyclonal antibody specific for human FGF-19. Standards and samples were pipetted into the wells and any human FGF-19 present is bound by the immobilized antibody.

Statistical analysis

Statistical analysis was done using IBM© SPSS© Statistics version 23 (IBM© Corp., Armonk, NY, USA). Numerical data were expressed as mean and standard deviation or median and range as appropriate. Qualitative data were expressed as frequency and percentage. Chi-square test (Fisher's exact test) was used to examine the relation between qualitative variables. The three groups were compared for quantitative data using ANOVA or Kruskal-Wallis test, followed by the appropriate post-Hoc test. Spearman-rho method was used to test the correlation between numerical variables. Sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), positive diagnostic likelihood ratio (PDLR), and negative diagnostic likelihood ratio (NDLR) were calculated for different index test cutoff values using the R package DTComPair.18 19. A p-value < 0.05 was considered significant.

Results

There was no significant difference between the three studied groups regarding age and sex (Table 1). Table 2 shows the baseline laboratory characteristics of the three studied groups. There was no statistically significant difference between the three groups regarding total leukocytic count and direct bilirubin. Significant intergroup differences existed between the three groups in hemoglobin concentration, platelet count, albumin, total bilirubin, ALT, AST, and INR. Hemoglobin concentration and albumin were significantly lower in the two patient groups than in the controls. Total bilirubin was

significantly higher in the HCC group than in the Cirrhosis and Control groups. ALT and AST were also significantly higher in the HCC group. INR was significantly higher in the HCC and Cirrhosis groups compared to the Control group. Creatinine and urea were significantly higher in the HCC group compared to the Control group (Table 2). Table 3 shows no significant difference between the HCC and Cirrhosis groups in the Child score (p=0.136).

The three markers, FGF-19, DCP, and AFP, were significantly different between the three groups, except that DCP was comparable between HCC and Cirrhosis groups (p=1.000) (Table 4). All individuals in the control group had FGF-19 levels below the minimum level in the HCC group. Thus, FGF-19 had 100% sensitivity and specificity in differentiating HCC from healthy controls. The diagnostic performance of the three markers for differentiating HCC from liver cirrhosis and differentiating cirrhosis from healthy controls using ROC curve analysis is shown in Table 5.

FGF-19 can discriminate between HCC and Cirrhosis groups at a 140.8 pg/mL cutoff with sensitivity and specificity of 81.8% and 87.9%, respectively. It discriminated cirrhosis from healthy Controls at a cutoff of 58.5 with sensitivity and specificity of 84.8% and 90.9%, respectively. DCP has poor diagnostic value for differentiating HCC from Cirrhosis. FGF-19 had significantly higher sensitivity and specificity than DCP. The sensitivity and specificity of FGF-19 were higher than AFP, trending toward statistical significance (p=0.095) (supplementary Table 6). On the other hand,

DCP had significantly higher sensitivity than FGF-19 for differentiating cirrhosis from healthy controls. Also, FGF-19 had comparable sensitivity and specificity for differentiating cirrhosis from healthy controls (supplementary Table 7). In the HCC group, FGF-19 was negatively correlated with serum creatinine and positively correlated with DCP. In the cirrhotic group, FGF-19 was positively correlated with platelet count and serum albumin and negatively correlated with INR and total and direct bilirubin (supplementary Table 8). There was no statistically significant relation between FGF-19 and Child score (p=0.627, supplementary Table 9).

A combined marker of FGF-19 ≥ 140.8 pg/mL or AFP ≥ 13.1 ng/mL improved the sensitivity of differentiating HCC from Cirrhosis, but it decreased specificity to 66.7%. A combined marker of FGF-19 ≥ 140.8 pg/mL or DCP ≥ 0.725 ng/mL improved the sensitivity of differentiating HCC from Cirrhosis, but it decreased specificity to 45.5%. A combination of the three markers when anyone is positive upgraded sensitivity to 93.9%, but the specificity was further dropped to 27.3% (supplementary Table 10).

Discussion

In recent years, novel biomarkers have been suggested to complement AFP and enhance the accuracy of HCC diagnosis [25]. In the current study, we aimed to evaluate the potential of FGF-19 as a new biomarker for HCC. The sensitivity and specificity of FGF-19 to discriminate between HCC and cirrhosis were 81.8%

Table 1. Demographic Characteristics among the Studied Groups

	HCC group n=38	Cirrhosis group n=38	Control group n=38	p-value
Age (years)	64.0±8.6	62.4±8.5	59.5±7.3	0.055
Male	30 (80.6%)	24 (63.9%)	22 (58.3%)	0.111

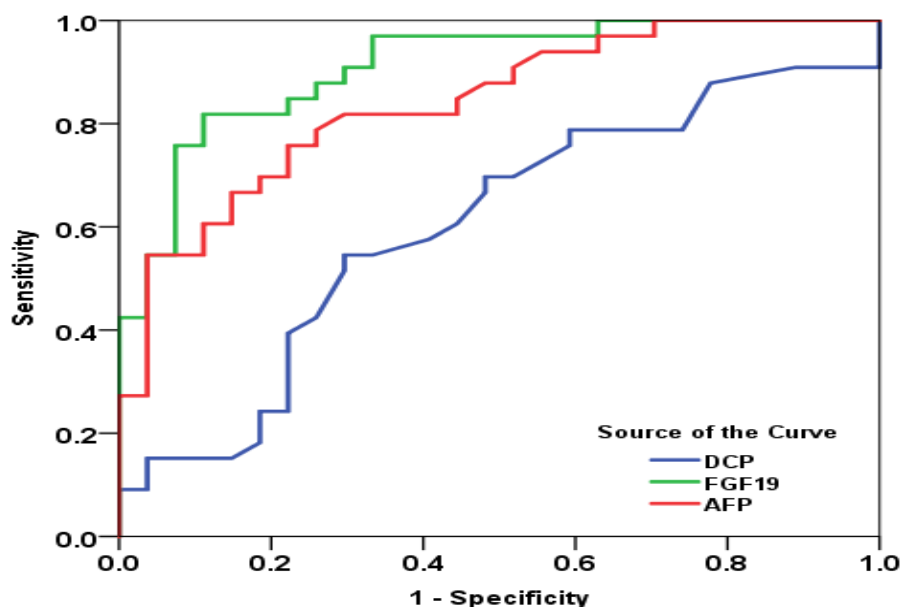Data are presented as mean±SD or number (%)

Figure 1. Receiver Operating Characteristic Curves for Calculating the Diagnostic Performance of FGF-19, AFP, and DCP for the Differentiation of HCC from Cirrhosis. FGF-19: Fibroblast growth factor 19, AFP: Alpha fetoprotein, DCP: Des-γ-carboxy prothrombin
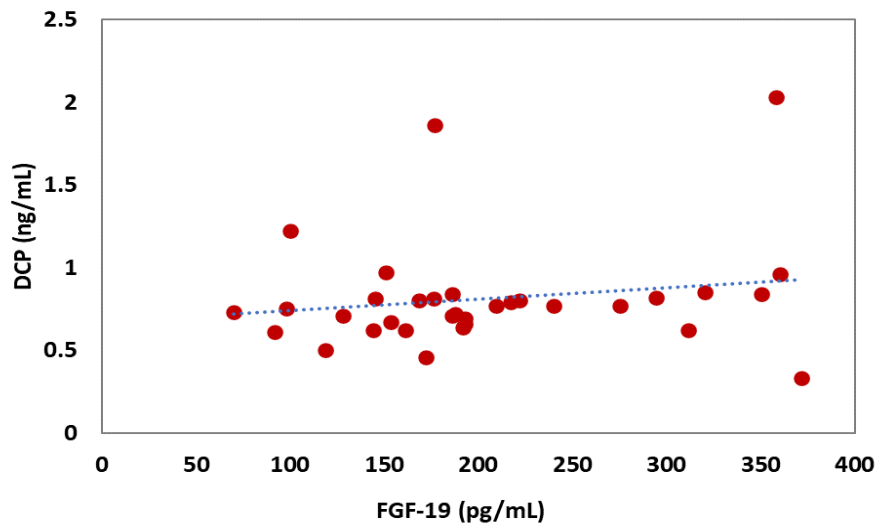
Figure 2. Scatter Diagram for the Correlation between Fibroblast Growth Factor 19 (FGF-19) and Des-γ-carboxy Prothrombin (DCP) in Patients with HCC

Table 2. Laboratory Characteristics of the Three Studied Groups

	HCC group n=38	Cirrhosis group n=38	Control group n=38	p-value	
Hemoglobin (gm/dL)	11.4±1.3	11.7±2.1	13.2±0.9	<0.001	p_1=0.659 p_2<0.001 p_3=0.010
TLC x10³	6.6±3.8	6.7±2.9	7.1±2.2	0.693	
Platelet count x10³	151±61	174±82	250±54	<0.001	p_1=0.467 p_2<0.001 p_3=0.001
Albumin (gm/dL)	3.23±0.49	3.51±0.53	4.54±0.62	<0.001	p_1=0.325 p_2<0.001 p_3=0.012
T. Bilirubin (mg/dL)	1.5 (0.8-4.3)	1.1 (0.1-3.3)	0.7 (0.1-1.8)	0.002	p_1=0.039 p_2=0.004 p_3=0.633
D. Bilirubin (mg/dL)	0.7 (0.1-2.2)	0.4 (0.1-1.6)	0.3 (0.2-0.7)	0.057	
ALT (IU/mL)	48 (12-198)	28 (12-139)	20 (12-28)	0.005	p_1=0.241 p_2=0.005 p_3=0.183
AST (IU/mL)	55 (18-271)	35 (1-78)	17 (10-30)	<0.001	p_1<0.001 p_2<0.001 p_3=0.078
INR	1.53±0.37	1.33±0.21	1.02±0.09	<0.001	p_1=0.161 p_2<0.001 p_3=0.001
S. creatinine (mg/dL)	1.16±0.39	0.99±0.33	0.83±0.27	0.003	p_1=0.341 p_2=0.002 p_3=0.300
B. urea (mg/dL)	32.5 (15-98)	24 (12-45)	18 (6-23)	0.001	p_1=0.148 p_2=0.001 p_3=0.095

Data are presented as mean±SD or median (range); TLC,Total leucocytic count; ALT, Alanine transaminase; AST, Aspartate aminotransferase; INR, International normalized ratio; p_1, HCC vs. Cirrhosis; p_2, HCC vs. Control; p_3, Cirrhosis vs. Control

Table 3. Child-Pugh Class of HCC and Cirrhosis Groups

		HCC group n=38	Cirrhosis group n=38	p-value
Child class	A	19 (52.8%)	27 (75.0%)	0.136
	B	11 (27.8%)	7 (16.7%)	
	C	8 (19.4%)	4 (8.3%)	

Data are presented as number (%)

Table 4. The Levels of Fibroblast Growth Factor 19, Des-γ-carboxy Prothrombin (DCP), and alpha-fetoprotein in the Three Studied Groups

		HCC group n=38	Cirrhosis group n=38	Control group n=38	p-value
FGF-19 (pg/mL)	Median	187	86.8	49.3	< 0.001
	Range	70.1-371.6	28.9-191.8	30.1-66.3	
DCP (ng/mL)	Median	0.77	0.73	0.49	< 0.001
	Range	0.33-2.03	0.55-1.12	0.43-0.95	
AFP (ng/mL)	Median	113	5	0.5	< 0.001
	Range	2.8- 2766	0.6-267	0.5-5.8	

FGF-19, Fibroblast Growth Factor-19; DCP, Des-γ-carboxy prothrombin; AFP, Alpha-fetoprotein

and 87.9%, respectively, at a cutoff of 140.8 pg/mL. At a cutoff of 58.5 pg/mL, its sensitivity and specificity to discriminate cirrhosis from healthy controls were 84.8% and 90.9%, respectively. FGF-19 had a higher sensitivity and specificity to AFP (75.8%, 77.7%, respectively) for diagnosis of HCC and cirrhosis, but the difference was not significant (p=0.527). DCP has poor diagnostic value for differentiating HCC from cirrhosis, significantly lower than FGF-19. Conversely, DCP had significantly higher sensitivity than FGF-19 for differentiating cirrhosis from healthy controls. A combined marker of FGF-19 ≥ 140.8 pg/mL or AFP ≥ 13.1 ng/mL improved the sensitivity of differentiating HCC from cirrhosis to 93.9%, but it decreased the specificity to 66.7%. A combined marker of positive FGF-19 or DCP had a better sensitivity but markedly low specificity for differentiating HCC from

Table 5. Diagnostic Performance of Fibroblast Growth Factor 19, Des-γ-carboxy Prothrombin (DCP), and Alpha-Fetoprotein for Diagnosis of HCC and Liver Cirrhosis

	Differentiating HCC from Cirrhosis		Differentiating Cirrhosis from Controls	
	Estimate	95%CI	Estimate	95%CI
FGF-19 (pg/mL)	≥ 140.8		≥ 58.8	
Sensitivity	81.80%	68.7%-95.0%	84.80%	72.6%-97.1%
Specificity	87.90%	76.7%-99.0%	90.90%	78.9%-100.0%
PPV	87.10%	75.3%-98.9%	93.30%	84.4%-100.0%
NPV	82.90%	70.4%-95.3%	80.00%	64.3%-95.7%
PDLR	6.75	2.66-17.15	9.33	2.47-35.26
NDLR	0.21	0.10-0.43	0.17	0.07-0.38
DCP (ng/mL)	≥ 0.725		≥ 0.585	
Sensitivity	57.60%	40.7%-74.4%	97.00%	91.1%-100.0%
Specificity	51.50%	34.5%-68.6%	90.90%	78.9%-100.0%
PPV	54.30%	37.8%-70.8%	94.10%	86.2%-100.0%
NPV	54.80%	37.3%-72.4%	95.20%	86.1%-100.0%
PDLR	1.19	0.75-1.88	10.67	2.84-40.04
NDLR	0.82	0.49-1.38	0.03	0.01-0.23
AFP (ng/mL)	≥ 13.1		≥ 1.77	
Sensitivity	75.80%	61.1%-90.4%	88.90%	77.0%-100.0%
Specificity	77.70%	62.1%-93.5%	90.90%	78.9%-100.0%
PPV	80.60%	66.7%-94.5%	92.30%	82.1%-100.0%
NPV	72.40%	56.1%-88.7%	87.00%	73.2%-100.0%
PDLR	3.4	1.64-7.09	9.78	2.59-36.90
NDLR	0.31	0.17-0.59	0.12	0.04-0.36

FGF-19, Fibroblast Growth Factor-19; DCP, Des-gamma-carboxy prothrombin; AFP, Alpha-fetoprotein; PPV, Positive predictive value; NPV, Negative predictive value; PDLR, Positive diagnostic likelihood ratio; NDLR, Negative diagnostic likelihood ratio

Cirrhosis. Notably, FGF-19 had 100% sensitivity and specificity in differentiating HCC from healthy controls. It worth noting the lowered specificity of combined markers is attributed to the number of cirrhotic patients having levels of AFP and DCP higher the selected cut-off values.

We found significantly higher FGF-19 levels in HCC patients than in cirrhotic patients and healthy controls. Also, the levels in cirrhosis patients were higher than controls, concordant with many previous studies [26–30]. Mohamed et al. [27] reported the same findings, while Maeda et al. [26] found no significant difference between the cirrhotic cases and controls. Li et al. [28] detected higher FGF-19 levels in serum and tissues in the HCC group compared to the control group. These results are consistent with Sun et al. [29] findings, who found higher FGF-19 levels in the diabetic HCC and HCC groups than in the diabetes and control groups. Sweed et al. showed overexpression of FGF19 protein in HCC patients compared to cirrhosis and healthy groups, irrespective of etiology, previous HCV treatment, or morphological variants of the disease [31]. These results suggest that FGF-19 may have a role in the pathogenesis of HCC.

FGF19 is secreted from the ileum and modulated its endocrine functions by binding to FGFR4 on hepatocytes [32]. FGF19 regulates liver BA and lipid metabolism and plays a crucial role in liver regeneration after partial hepatectomy [33]. FGF19 increase stimulates hepatocellular protein synthesis and proliferation. However, the signaling pathways for FGF19-dependent tumorigenesis are not yet clear. Extracellular signal-regulated protein kinase (ERK) and β-catenin have been linked to hepatocyte proliferation, survival, migration, invasion, and angiogenesis [34].

FGF19 regulates β-catenin during epithelial-mesenchymal transition (EMT) in human colon cancer cells. FGF19 is significantly increased and negatively associated with E-cadherin expression in HCC tissues [22]. It also activates the stress-regulated transcription factor called nuclear factor (erythroid-derived 2)-like 2 in HCC cells. However, in a subtype of HCC patients, β-catenin can play a role in HCC without FGF19 amplification [35]. FGF19 overexpression regulates endoplasmic reticulum (ER) stress, which promotes tumor cell proliferation via activation of the FGFR4-GS3Kβ-Nrf2 signaling pathway [36]. FGF19 increases IL-6 production in a mouse model, followed by STAT3 activation in hepatocytes [37].

FGF19 can activate Ras and ERK, which play critical roles in HCC development [38]. Latasa et al. found that FGF19 induced amphiregulin expression by activating β-catenin signaling in HCC. Amphiregulin is a ligand of the epidermal growth factor receptor, which is crucial for the proliferation, survival, and resistance of HCC [39]. Additionally, FGF19/FGFR4 binding contributed to the regulation of various features of cancer [19].

Therefore, FGF19 could be used as a serum biomarker of HCC. The results of the present study support this notion, as its sensitivity and specificity for detecting HCC in cirrhotic patients were 81.8% and 87.9%, respectively. The sensitivity was higher than that of AFP (p=0.527),

and their combination had 93.3% sensitivity.

Previous studies reported various diagnostic accuracy values with different cutoffs. For example, Mohamed et al. [27] found a sensitivity of 100% and specificity of 90% at a cutoff > 180 pg/mL. A markedly lower sensitivity of 53.2% and higher specificity of 95.1% were reported by Maeda et al. [26] at a more conservative cutoff point of 200 pg/mL. We can conclude that FGF-19 appears to be a possible noninvasive serum biomarker for HCC. Its combination with AFP improves the sensitivity for the detection of HCC.

Author Contribution Statement

All authors have substantially contributed to the conception and design, acquisition of data, data analysis, and interpretation. All authors have agreed on the content of the manuscript. Ahmed Hamed Rashad, MD: manuscript writing. Mohamed Oraby, MSc: Patient recruitment and follow-up. Amaal A. Abdelaal, MD: Performed the laboratory studies and revised the data. Amel E Salem, MD: Revised and prepared data for manuscript writing. Rabab Mohamed Maher, MD: Patient recruitment and follow-up. Mahmoud Abdo Abdelalem, MD: Study design, conception, and manuscript revision. I declare that no financial or editorial assistance has been received by any of the authors to support the research project and/or preparation of the article.

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