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RESEARCH ARTICLE

Fibroblast Growth Factor 19 is an Independent Prognostic Parameter for Recurrence of Hepatocellular Carcinoma

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ABSTRACT

Background: Hepatocellular carcinoma (HCC) remains a common cancer associated with a high mortality rate. Genes that drive HCC development accumulate randomly that could be a target for therapeutic management. In large-scale studies, the dysregulation of fibroblast growth factors (FGFRs) was detected in over 7% of cancers and served as an oncogenic signalling pathway. Amplification of FGF19 was found to be associated with the development of HBV and NAFLD related HCC with an ongoing clinical trial for targeting this pathway. Aim: We aimed to examine FGF19 expression profile in HCV related HCC and to compare its level of expression within the two main morphological patterns. **Materials and Methods:** This was a retrospective, case-control study that included 76 HCC cases and 53 adjacent non-tumour liver, 20 cirrhosis and 20 normal liver tissue. FGF19 expression was assessed in the studied groups immunohistochemically. . **Results:** FGF19 was expressed in 26.3% of HCC cases with no significant difference in its expression between the two HCC subgroups (P=0.073). The expression of FGF19 was not differed in the HCC group according to the aetiology (*P*=0.605), prior HCV treatment (*P*=0.912) or background liver cirrhosis (*P*=0.931). The only factor that associated with increased FGF19 expression was the marked inflammatory activity of the liver (*P*=0.026). COX regression analysis revealed that FGF19 was the single independent factor affecting tumor recurrence (*P*=0.04). **Conclusions:** Given its common expression in HCC independent of etiological, background liver or morphological subtypes, FGF19 could be used as an indicator to predict tumor recurrence in HCC.

Keywords: FGF19, HCC, Macrotrabecular massive, NOS, Recurrence

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INTRODUCTION

Hepatocellular carcinoma (HCC) remains the most common cancers related to a high mortality rate (Ferlay et al., 2015, Bray et al., 2018). In several countries, HCC is considered the leading cause of cancer-related death in both men and women (Sung et al., 2021). The risk factors vary according to geographic distribution with chronic hepatitis B (HBV) and hepatitis C virus (HCV) infection account for 56% and 20% of HCC related deaths worldwide, respectively. On the other hand, the non-viral risk factors including non-alcoholic fatty liver disease have become the leading cause of HCC in Western countries (Kulik and El-Serag, 2019).

Despite, the emerging of direct-acting antiviral drugs (DAAs) in eradication of HCV infection, the incidence of HCC may persist post treatment for the years particularly in cirrhotic patients (Ioannou et al., 2019). HCC is a complex multistep process that entails an interplay of many factors including interactions between viral and non-viral risk factors, environmental and immunological factors on a genetic predisposition patient (Llovet et al., 2021).

Although genes that drive HCC development accumulate randomly, precise molecular HCC subclasses could be related to specific genes profiles that could target therapeutic management (Llovet et al., 2018).

The molecular classifications of HCC vary based on etiological, genetic alteration and morphological pattern which may impact the prognostic parameters (Calderaro et al., 2019). Two broad molecular classes of HCC, the proliferation and non-proliferation classes have been reported. The proliferation class divides into two main HCC morphological variants; fibrolamellar (FLC) and macrotrabecular massive type while the non-proliferation class includes HCC, not otherwise specified (NOS). Based on genetic changes, the proliferation class shares fibroblast growth factor 19 (FGF19) amplification and has a more aggressive course and links mainly to the HBV infection (Llovet et al., 2021).

The FGFs family is composed of a large members of growth factors that function through binding to a couple of transmembrane tyrosine kinase, FGFRs 1-4 distributed in organs (Ornitz and Itoh, 2001, Itoh and Ornitz, 2011). Under physiological conditions, FGFs–FGFRs modulate many biological functions; cell proliferation and differentiation, embryonic development, and tissue repair (Wilkie et al., 1995). FGF19 is secreted from the ileum through activation by the transcription factor Farnesoid X receptor (FXR) (Toyoda et al., 2015, Chiang and Ferrell, 2018). Upon activation, FGF19 binds to hepatocyte FGFR-4 and plays major roles in bile acid (BA) synthesis, gluconeogenesis, glycogen synthesis, and protein synthesis (Ashby et al., 2018). FGF19 is not expressed in normal hepatocytes, whereas the bile duct regulates the function of FGF19 in liver cholestasis (Somm and Jornayvaz, 2018).

It has been found in large-scale high-throughput studies that FGFRs dysregulation is over 7% of cancers (Helsten et al., 2016). FGFR dysregulation is considered as an oncogenic signalling pathway in various human cancers (Vergote et al., 2013, Babina and Turner, 2017). Amplification of FGF19 was found to be significantly related to the development of HBV and NAFLD related HCC (Ahn et al., 2014, Cui et al., 2018). FGF19 functioned through autocrine fashion to reportedly activate FGF19/FGFR4 signalling and contribute to HCC development and progression (Zhao et al., 2016). Therefore, FGF19 and its target receptor FGF4 could have a potential therapeutic role in many cancer (Lang and Teng, 2019, Maeda et al., 2019). However, data on the role of FGF19 on HCV related HCC is not well elucidated. Also, the expression of FGF19 in the HCC NOS is not yet clear.

In this study, we aimed to examine the expression level of FGF19 protein in HCC and correlate its expression with clinicopathological and survival data. we also aimed to compare FGF19 protein expression within the two mains morphological-molecular pattern of HCC.

MATERIALs AND METHODS

This study is a retrospective, case-control study performed on formalin-fixed, paraffin-embedded (FFPE) specimens included 76 HCC cases and 53 adjacent non-tumour liver, 20 cirrhosis and 20 normal liver tissues obtained from the donors of liver transplantation as normal controls. Samples were collected as a part of the standard clinical management of the patients. For HCC cases, clinical and laboratory data were collected from patients' medical records. All cases were assessed, evaluated, and underwent surgery in the Hepatobiliary Surgery Department at National Liver Institute between the period 2018-2020. Ethical committee approval number was 00245/2021.

All surgeries were done under general anaesthesia in hepatopancreaticobiliary surgery (HPBS) Department (Dr. Mohamed Taha a HPBS consultant participated in most of surgeries). The technique was as follows the patient lay in dorsal position, abdominal exploration was done through supraumblical incision with right sided extension (inverted L shaped incision), hepatectomies were major resections (anatomical resection) in 49 % of cases (26% formal right hepatectomy, 13% formal left hepatectomy and 10% left lateral hepatectomy), and minor resections (non-anatomical resections) in 51 % of cases (36% right hepatic lobe, 11% left hepatic lobe, and 4% hepatic bilobar). Overall survival (OS) data was calculated in months from the date of diagnosis to the time of death or the date of the last follow up visit. HCC cases were assessed by hepatopathologist according to the 5th edition of WHO of tumours of the digestive system and the 8th edition of AJCC staging system (Amin et al., 2017, Nagtegaal et al., 2020).

Immunohistochemistry (IHC)

The tissue microarray technique was performed on the HCC cases and the adjacent non-tumor liver tissues according to the previously published protocol (Abdel-Rahman et al., 2014). At least two representative cores from each tumor and one core from matching non-tumor tissue were included.

Rabbit polyclonal fibroblast growth factor 19 (FGF19) antibody (Ref; ab225942) was obtained from Abcam, Cambridge, UK. High PH Tris-EDTA antigen retrieval solution (Dako, Ref K8000, Glostrup, Denmark) was carried out for 20 minutes of heating followed by cooling for 20 minutes at room temperature. FGF19 was diluted in DAKO antibody diluent at (1:200) and incubated overnight at 4^{o}C. Detection of the immunostaining was carried by using the Envision™ FLEX/HRP detection system (DAKO A/S, Glostrup, Denmark) with the chromogen 3-diaminobenzidine (DAKO). For optimization of the immunohistochemistry protocol, human intestinal tissue was used as a positive control. Also, a negative control by the omission of the primary antibody was used for each run.

FGF19 IHC Assessment

Positive FGF19 expression should be considered if any brownish cytoplasmic staining of hepatocytes was observed. Two methods of assessment were applied according to previously mentioned protocols. First based on multiplication of the staining intensity and the positive cell percentage. Scoring for the staining intensity was as following (0) for negative staining, (1) for weak staining, (2) for moderate staining and (3) for strong staining, and scores of the percent of positive hepatocytes were defined as follows: 0, <10%; 1, 10–30%; 2, >30–50%; and 3, >50% positive cells. Using a score of 4 as a cut-off, we defined score ≥4 was as positive FGF19 and score <4 was as negative FGF19 expression (Schulze et al., 2015). The other method of assessment using a Histoscore (H score) system that calculated by multiplying staining intensity (0-3) by the percentage of stained cells with a final score ranging from 0 to 300 (Zhang et al., 2015).

Statistical Analysis

The analysis of the data was done by using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Additionally, The Kolmogorov-Smirnov was used for the verification of the normality of the distribution of variables. Chi-square test (Fisher or Monte Carlo) was used for comparisons between groups for categorical variables. Furthermore, the Student t-test was used to compare two groups for normally distributed quantitative variables, in addition to Mann Whitney and Kruskal Wallis tests were used for not normally distributed quantitative variables test and Post Hoc test (Dunn's for multiple comparisons test) for pairwise comparison. Regarding the correlation between quantitative variables, the Spearman coefficient was used and McNemar Test used to analyse the significance between the different stages. Judgement of the significance of the obtained results was at the 5% level. COX regression analysis was used to investigate the effect of several parameters on the HCC recurrence and survival. Kaplan Meier analysis was done to assess the parameters affecting the OS and disease free survival (DFS).

RESULTS

The Clinicopathological Data of HCC Cases

The median age for HCC cases was 59 years old with male predominance (80.3%). HCV aetiology was reported in 92.5% of cases in which 58.6% received prior DAAs, 15.5% received prior interferon and 25.9% had no treatment. The median serum AFP level was 33 ng/dl. Grossly, 72 % of HCC cases were solitary with the median tumour size was 4.3 cm arising on top of liver cirrhosis in 65.8% of cases. Three morphological patterns were included, HCC, NOS (81.6%), macrotrabecular massive (14.5%), and FLC (3.9%). The pathological stage was early in 86.8% of cases and only 16.7% of cases developed tumour recurrence. We further compared the two main HCC groups: HCC, NOS (as an example of class B) and macrotrabecular massive variant (as an example of class A) regarding the clinicopathological parameters. The macrotrabecular variant was significantly associated with the higher serum AFP level and larger tumour size compared to NOS group (P=0.048 and P=0.04, respectively). In addition, the macrotrabecular type showed significant clear cell changes (P=0.042). The comparison between HCC, NOS and macrotrabecular subgroups was illustrated in Table 1.

Protein expression of FGF19 in the Studied Cases

The detailed FGF19 expression in the studied groups was summarized in table 2. All normal liver tissue cases showed negative FGF19 expression. On the other hand, FGF19 was expressed in 10%, 11.3% and 26.3% of liver cirrhosis, adjacent non-tumour liver and HCC cases, respectively. In HCC subgroups, FGF19 was expressed in 24.2% and 27.3% of NOS and macrotrabecular massive types, respectively. Also, the three FLC cases (100%) were positive for FGF19 expression with the median H score was 150 and mean H score expression was 126.7±87.4, Figure 1.

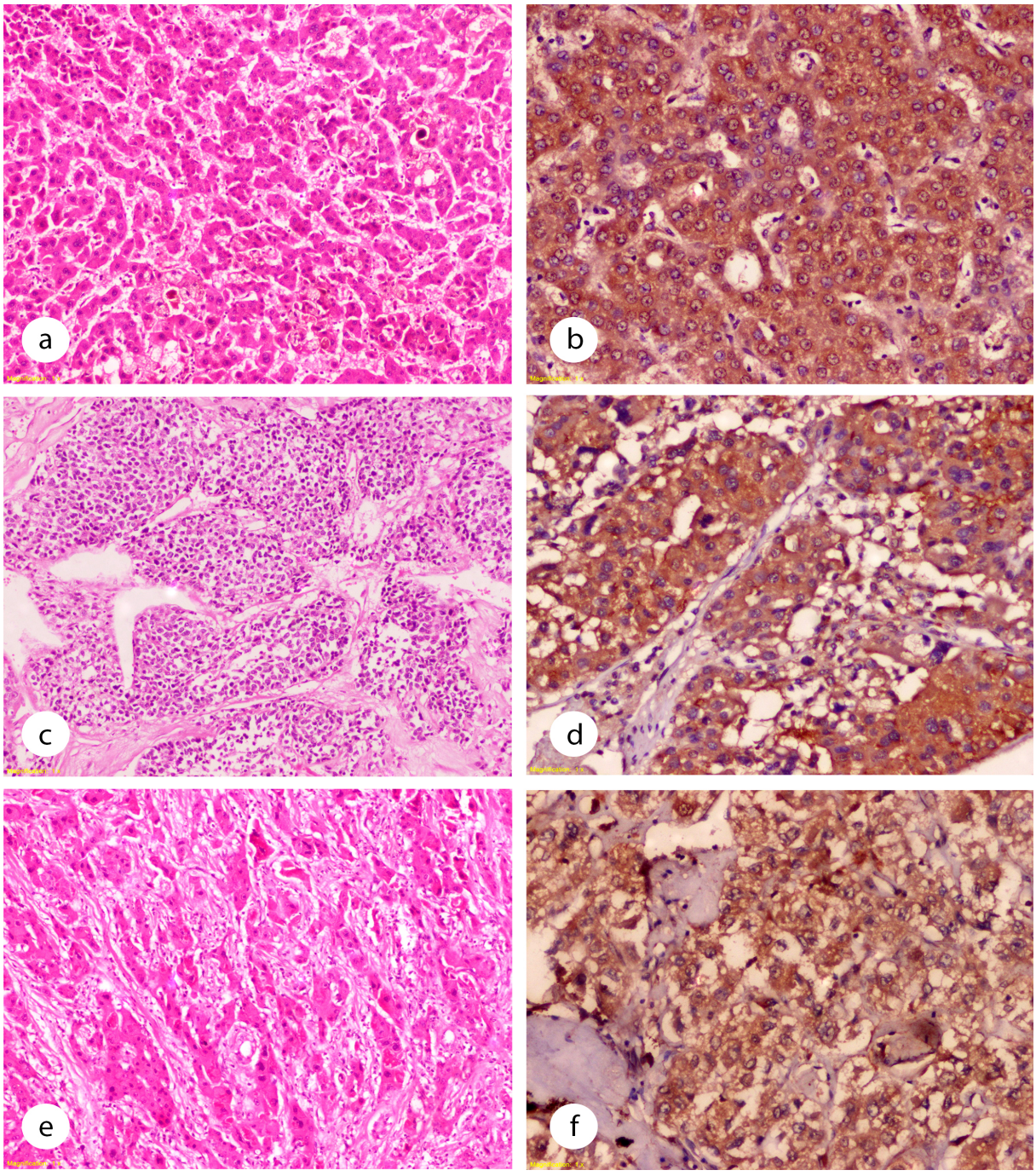

Figure 1. Expression of FGF19 in the different HCC morphological patterns. a) A case of HCC NOS showed an acinar and trabecular pattern with bile formation (H&E x100). b) FGF19 showed strong cytoplasmic expression in HCC NOS (IHC x200). c) A case of HCC macrotrabecular massive showed trabecular thickness >10 cells (H&E x100). d) FGF19 showed strong cytoplasmic expression in HCC macrotrabecular massive type. e) A case of fibrolamellar HCC showed hepatocytes with abundant eosinophilic cytoplasm separated by collagen bundles (H&E x100). f) FGF19 showed strong cytoplasmic expression in fibrolamellar HCC (IHC x200).

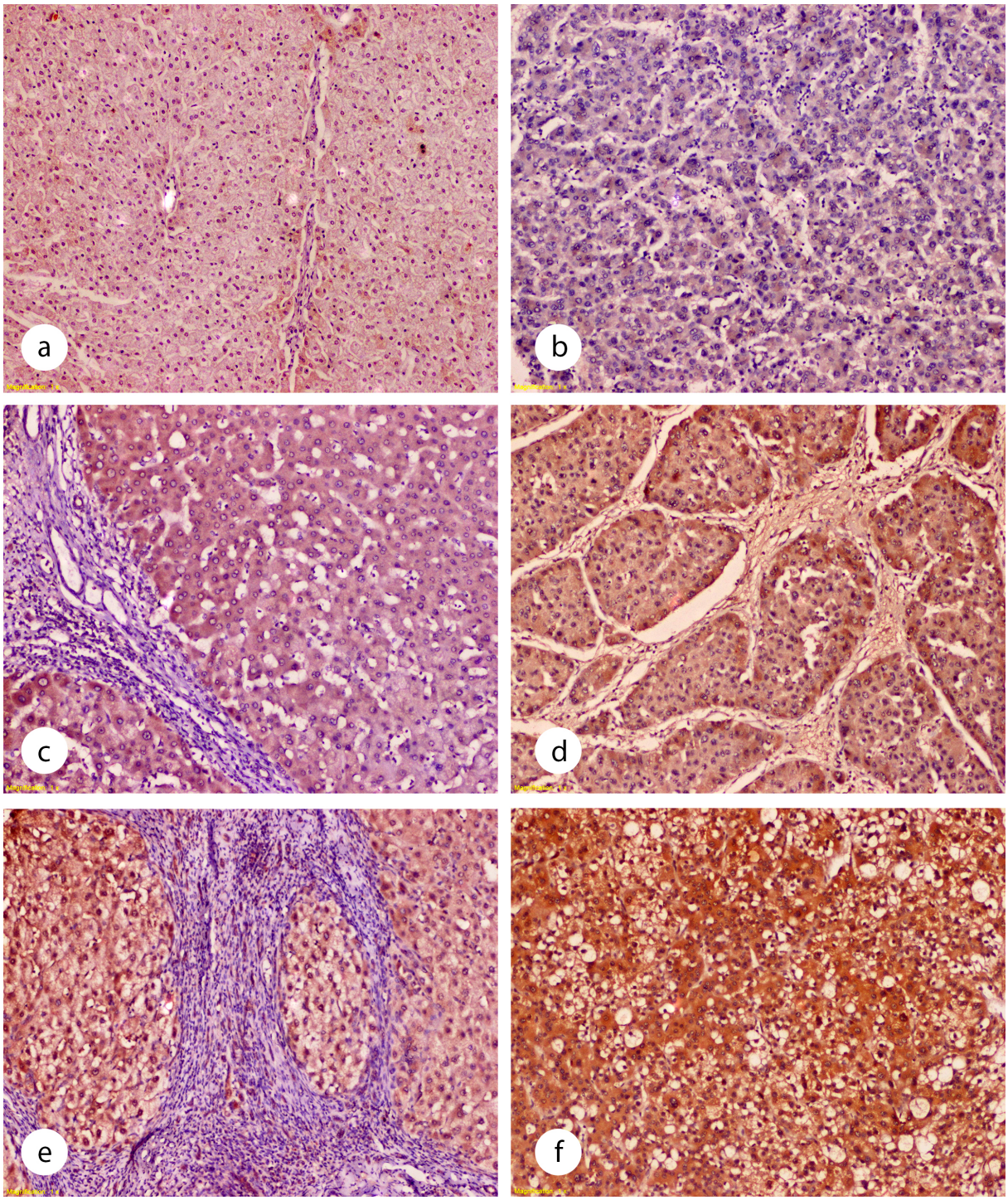

Figure 2. Expression of FGF19 in the studied groups. a) A case of normal liver tissue showed negative FGF19 expression. b) A case of HCC showed negative FGF19 expression. c) A case of non-tumour liver cirrhosis with mild inflammatory activity showed negative FGF19 expression. d) The corresponding HCC showed mild FGF19 expression. e) A case of non-tumour liver cirrhosis with severe inflammatory activity showed mild FGF19 expression,.f) The corresponding HCC showed strong FGF19 expression (IHC x100).

There is no significant difference between the liver cirrhosis and the normal liver tissue groups regarding FGF19 H score expression (*P*=0.074). Moreover, there was significant overexpression of FGF19 in HCC group compared to the normal and cirrhosis groups (*P*<0.001 and *P*=0.005, respectively). However, there was no significant difference between HCC and the adjacent non-tumour liver regarding FGF19 H score expression (*P*=0.932). Similarly, there was no significant difference between the two HCC subgroups regarding FGF19 H score expression (*P*=0.073), Figure 2.

Association of FGF19 Expression and the Clinicopathological Parameters of HCC Group

There is significant association between positive FGF19 expression and high serum AFP level and tumour necrosis (*P*=0.041 and *P*=0.009, respectively) and trend of significance between high FGF19 expression with the female gender and positive LVI (*P*=0.057 and *P*=0.071, respectively). There is no significant difference between HCC arising on a background of liver cirrhosis compared to non-cirrhosis regarding FGF19 expression (*P*=0.931), the grade of the inflammatory activity of background liver was significantly associated with positive FGF19 expression on HCC (*P*=0.026), Table 3.

COX Regression and Kaplan Meier Analysis for Factors affecting Survival and Recurrence

COX regression analysis for factors affecting HCC recurrence revealed that positive FGF19 expression was the only independent factor that predicts the tumour recurrence (Hazard ratio was 6.555, 95% CI (1.092–39.342) and *P*=0.04).

However, there was no impact of FGF19 expression on the patient's mortality (Hazard ratio was 0.986, 95% CI (0.358 – 2.714) and *P*=0.978), Table 4. Also, Kaplan Meier analysis for assessing the parameters affecting DFS showed a significant association of FGF19 expression and the high recurrence rate (mean 38.33, log-rank 5.707 and P=0.017). On the other hand, Kaplan Meier analysis for the parameters affecting OS showed no significant association with any of the clinicopathological parameters or FGF19 expression.

DISCUSSION

The current study aimed to evaluate FGF19 protein expression in HCC cases and to verify its expression in different HCC morphological subgroups, background liver cirrhosis and HCV aetiology. The prognostic role of FGF19 expression was evaluated in context with tumour recurrence and patients' survival. Our results showed overexpression of FGF19 protein in HCC cases compared to normal and cirrhosis groups. Furthermore, the FGF19 expression was not peculiar to a certain aetiology, prior HCV treatment, background liver cirrhosis or certain morphological variants of HCC. The only factor impact FGF19 expression was the marked inflammatory activity of the background liver. FGF19 overexpression in HCC cases was the only independent factor that predicts tumour recurrence.

FGF19 was not expressed on the normal liver tissue group and this is consistent with its physiological function. FGF19 secreted from the ileum and modulated its endocrine functions through binding to hepatocytes FGFR4 to regulate BA metabolism (Wunsch et al., 2015). In addition, FGF19 expression in the liver cirrhosis group has not differed significantly from the normal liver group; however, only a trend of FGF19 overexpression was noticed in the cirrhosis group. Previous studies found an association between elevated FGF19 levels and the severity of hepatic fibrosis and cirrhosis of different aetiologies. FGF19 exerted its fibrogenesis through either regulation of BA metabolism or activation of hepatic stem cell (Schumacher and Guo, 2016). On contrary, other studies reported the beneficial and possible therapeutic role of FGF19 on liver regeneration post-hepatectomy to maintain normal hepatocytes proliferation (Alvarez-Sola et al., 2018). Therefore, the role of FGF19 in either hepatocyte regeneration or hepatic fibrosis is still a matter of debate necessitating further studies. In the current study, FGF19 was overexpressed in the HCC group compared to the normal and cirrhosis groups. FGF19 downregulated several pathways exerting a dual effect on enhancing hepatocytes proliferation and metabolic alteration driving HCC development (Miura et al., 2012, Massafra et al., 2017). In addition, FGF19/FGFR4 binding contributed to the regulation of different hallmarks of cancer (Gross et al., 2015, Raja et al., 2019). FGF19 protein was expressed in nearly 25% of HCC cases similar to two previous studies that found the frequency of FGF19 expression in HCC was 14-20% and 27% (Sawey et al., 2011, Kim et al., 2019).

Contrary to our results, previous studies reported a high frequency of FGF19 expression in HCC cases 46% and 100% (Desnoyers et al., 2008, Miura et al., 2012).

Table 1. Comparison between HCC subgroups according to the clinicopathological parameters (n=73)

	NOS (n= 62)	Macrotrabecular (n= 11)	Test of Significance
Age			
Mean ± SD.	58.5 ± 6.8	54.9 ± 14.3	t=1.337 0.185
Sex			
Male	10 (16.1%)	3 (27.3%)	χ^2=0.793
Female	62 (100%)	11 (100%)	^{FE}p=0.373
Etiology			
Non-viral	2 (3.7%)	1 (9.1%)	χ^2=0.602
HCV	52 (96.3%)	10 (90.9%)	^{FE}p=0.432
Previous HCV treatment			
No	13 (26.5%)	2 (22.2%)	χ^2=0.647
DAA	29 (59.2%)	5 (55.6%)	^{MC}p=0.770
Interferon	7 (14.3%)	2 (22.2%)	
AFP			
<200	32 (72.7%)	3 (33.3%)	χ^2=5.170*
>200	12 (27.3%)	6 (66.7%)	^{FE}p=0.048*
Focality			
Solitary	44 (71%)	8 (72.7%)	χ^2=0.014
Multiple	18 (29%)	3 (27.3%)	^{FE}p=1.000
Size			
Median (Min. – Max.)	4 (1.5 – 18)	6.5 (3 – 17)	U=209.0* 0.040*
Clear cell changes			
Median (Min. – Max.)	0 (0 – 90)	5 (0 – 70)	U=226.0* 0.042*
TILs			
Median (Min. – Max.)	5 (0 – 40)	5 (0 – 30)	U=325.50 0.805
Necrosis			
Median (Min. – Max.)	0 (0 – 75)	0 (0 – 60)	U=335.0 0.903
Stage			
I	18 (29%)	1 (9.1%)	χ^2=2.243
II	35 (56.5%)	9 (81.8%)	^{MC}p=0.342
III	9 (14.5%)	1 (9.1%)	
LVI			
No	28 (45.2%)	3 (27.3%)	χ^2=1.224
Yes	34 (54.8%)	8 (72.7%)	^{FE}p=0.335
Liver			
Non-cirrhosis	20 (32.3%)	3 (27.3%)	χ^2=0.108
Cirrhosis	42 (67.7%)	8 (72.7%)	^{FE}p=1.000
Recurrence (n= 30)			
No	20 (83.3%)	5 (83.3%)	χ^2=0.0
Yes	4 (16.7%)	1 (16.7%)	^{FE}p=1.000

χ^2: Chi square test, MC: Monte Carlo, FE: Fisher Exact t: Student t-test, U: Mann Whitney test, p: p value for comparing between the studied groups, *: Statistically significant at p ≤ 0.05

On the other hand, a large cohort study performed by Kang et al. reported the amplification of FGF19 in 5.15% of HCC cases (Kang et al., 2019). Even more, the frequency of FGF19 copy number (CN) gain was identified in 7.8% of liver cancer based on the TCGA liver cancer dataset. Kaibori et al. associated this discrepancy with the lack of correlation between FGF19 CN gain and the mRNA and protein (Kaibori et al., 2016). However, previous studies showed a good correlation between FGF19 CN and protein expression (Sawey et al., 2011, Miura et al., 2012). Another explanation is the upregulation of FGF19 protein expression via the epigenetic changes without FGF19 gene amplification which was reported in nearly 23% of HCC cases (Hoeflich et al., 2015). In the present study, there was no significant difference between HCV related and non-viral related HCC regarding FGF19 expression consistent with Miura et al. data (Miura et al., 2012).

Table 2. Comparison between the studied groups according to the FGF19 expression

	Normal (n=20)	Cirrhosis (n=20)	HCC (n=76)	Non tumor (n=53)	p
FGF19 expression					
Negative	20 (100%)	18 (90%)	56 (73.7%)	47(88.7%)	p_1=0.147 p_2=0.010*
Positive	0 (0%)	2 (10%)	20 (26.3%)	6(11.3%)	p_3=0.147 p_4=0.227
FGF19 H score					
Mean ± SD.	12.5 ± 25.1	37.5 ± 51.2	83.7 ± 81.2	76 ± 68.3	p_1=0.074 p_2<0.001*
Median (Min. – Max.)	0 (0 – 100)	30 (0 – 200)	60 (0 – 300)	60 (0 – 300)	p_3=0.005* p_4=0.932

	HCC subgroups		
	NOS (n= 62)	Macrotrabecular (n= 11)	p
FGF19 expression			
Negative	47 (75.8%)	8 (72.7%)	^{FE}p=1.000
Positive	15 (24.2%)	3 (27.3%)	
FGF19 H score			
Mean ± SD.	81.3 ± 78.7	85.5 ± 97.6	P=0.073
Median (Min. – Max.)	60 (0 – 300)	50 (0 – 300)	

p_1: p value for comparing between Normal and Cirrhosis, p_2: p value for comparing between Normal and HCC, p_3: p value for comparing between Cirrhosis and HCC, p_4: p value for comparing between tumor and non-tumor HCC, *: Statistically significant at p ≤ 0.05

A significant association of FGF19 overexpression was reported in HBV and NAFLD related HCC (Ahn et al., 2014, Cui et al., 2018). In addition, FGF19 was overexpressed in HCV related HCC (Kaibori et al., 2016). Therefore, FGF19 is an important driver of HCC development regardless of the aetiology. Similarly, the present study showed no significant difference regarding FGF19 expression in HCC raised on a background liver cirrhosis or non-cirrhosis. Kang et al. has found no significant association bewteen background liver cirrhosis and FGF19 amplification (Kang et al., 2019).

However, the expression was significantly higher in HCC raised on background liver of severe inflammatory activity. The impact of inflammation on FGF19 expression was reported in NAFLD related HCC through induction of oxidative stress, enhance fibrosis and mutational changes (Siegel and Zhu, 2009, Starley et al., 2010, Schreuder et al., 2010). Furthermore, Wong et al. hypothesized an increased number of NAFLD related HCC as a result of the inflammatory changes (Wong et al., 2014). These findings could indicate significant role of FGF19 in hepatocarcinogenesis irrespective of the predisposing factors. Sawey et al. reported a significant association of FGF19 expression with HCC macrotrabecular massive type (Sawey et al., 2011). In addition, the molecular classification of HCC found that FGF19 amplification was characteristic in the macrotrabecular morphological pattern and associated with a worse prognosis (Llovet et al., 2021). However, our data showed no significant difference regarding FGF19 in both HCC subgroups. In addition, Llovet et al. reported FGF19 amplifications in mixed FLC/HCC which was observed in our study. The three cases of FLC were positive for FGF19 and showed strong expression. FLC showed an enriched cancer stem cells population (Oikawa et al., 2015).
The association of FGF19 and FLC could be explained by the role of HSC/progenitor cells induced by FGF19 activation (Seitz et al., 2020).

In the present study FGF19 overexpression in HCC cases was significantly associated with high serum AFP, tumour necrosis, and increase risk of HCC recurrence. Our data were in context with the previous studies that correlated the high FGF19 expression with the poor HCC prognostic parameters (Sawey et al., 2011, Kan et al., 2013, Kang et al., 2019). The activation of the FGF19/FGFR4 system-induced HCC progression through enhancement of cell proliferation, invasion stimulation and inhibit apoptosis (Mitsuhashi et al., 2003, Miura et al., 2012).

Table 3. The association between FGF19 expression and clinicopathological parameters in HCC group (n= 76)

	FGF19 expression		Test of sig.
	Negative (n= 56)	Positive (n= 20)	p-value
Age			
Mean ± SD.	56.9 ± 9.9	55.8 ± 13.6	t=0.387 p=0.700
Sex			
Male	48 (85.7%)	13 (65.0%)	χ^2=3.992
Female	8 (14.3%)	7 (35.0%)	FE p=0.057
Etiology			
Non-viral	3 (6.1%)	2 (11.1%)	χ^2=0.474
HCV	46 (93.9%)	16 (88.9%)	FE p=0.605
Previous HCV treatment			
No	12 (27.3%)	3 (21.4%)	χ^2=0.286
DAA	25 (56.8%)	9 (64.3%)	MC p=0.912
Interferon	7 (15.9%)	2 (14.3%)	
AFP			
Median (Min. – Max.)	23.3 (1.5 – 9071.0)	300.0 (13.3 – 2527.0)	U=161.0* p=0.041*
Focality			
Solitary	38 (67.9%)	17 (85.0%)	χ^2=2.166
Multiple	18 (32.1%)	3 (15.0%)	p=0.141
Size			
Median (Min. – Max.)	4.0 (1.5 – 18.0)	5.3 (2.5 – 15.0)	U=456.0 p=0.216
Clear cell changes			
Median (Min. – Max.)	0.0 (0.0 – 90.0)	2.5 (0.0 – 70.0)	U=457.0 p=0.160
TILs			
Median (Min. – Max.)	5.0 (0.0 – 40.0)	5.0 (0.0 – 30.0)	U=508.50 p=0.528
Necrosis			
Median (Min. – Max.)	0.0 (0.0 – 75.0)	0.0 (0.0 – 70.0)	U=395.50* p=0.009*
Liver			
Non-cirrhosis	19 (33.9%)	7 (35.0%)	χ^2=0.008
Cirrhosis	37 (66.1%)	13 (65.0%)	p=0.931
Liver activity			
Low	33 (58.9%)	6 (30.0%)	χ^2=4.937*
High	23 (41.1%)	14 (70.0%)	p=0.026*
Stage			
I	18 (32.1%)	2 (10.0%)	χ^2=4.652
II	30 (53.6%)	16 (80.0%)	p=0.098
III	8 (14.3%)	2 (10.0%)	
LVI			
No	27 (48.2%)	5 (25.0%)	χ^2=3.258
Yes	29 (51.8%)	15 (75.0%)	p=0.071

χ^2: Chi square test, MC: Monte Carlo, FE: Fisher Exact, t: Student t-test, U: Mann Whitney test, p: p value for comparing between the studied groups, *: Statistically significant at p ≤ 0.05

FGF19 is thought to be a potential therapeutic target because it plays an important role in the proliferation of both tumour cells and endothelial cells (Repana and Ross, 2015). Tumours with FGF19 amplification are reportedly related to cellular sensitivity to FGFR inhibitors, in keeping with preclinical studies (Guagnano et al., 2012). Multikinase inhibitors like sorafenib and Lenvatinib also showed to be more effective in HCC patients with higher serum FGF19 levels (Kaibori et al., 2016, Casadei Gardini et al., 2019). The FGF19/FGFR4 axis, on the other hand, leads to sorafenib resistance (Gao et al., 2017). Both FGF19 and FGFR4 depletion enhances tumour cell sensitivity to sorafenib, resulting in increased apoptosis and decreased viability. Ponatinib, a third generation multitarget kinase inhibitor, has been shown to suppress FGF19/FGFR4 signalling and reverse sorafenib sensitivity (Gao et al., 2017). Furthermore, FGF19 signalling through the FGFR4/-klotho receptor complex has been shown to be a key driver of hepatocellular carcinoma growth and survival, making selective FGFR4 inhibition an appealing therapeutic option.

Table 4. Univariate regression analysis for the parameters affecting morality and recurrence.

Variables	Mortality (20/56)		Recurrence (5/30)	
	p	HR (95%C.I)	p	HR (95%C.I)
Viral etiology	**0.625**	0.694(0.160 – 3.001)	**0.669**	22.798(0.0 – 38517890.82)
AFP	**0.220**	1.0(1.0 – 1.0)	**0.657**	1.0(0.999 – 1.001)
Size	**0.639**	0.965(0.831 – 1.102)	**0.900**	0.984(0.770 – 1.259)
LVI	**0.245**	1.726(0.687 – 4.335)	**0.606**	1.600(0.267 – 9.580)
Variants (Macrotrabecular)	**0.635**	1.305(0.435 – 3.914)	**0.945**	1.081(0.121 – 9.672)
Stage (late)	**0.907**	0.916(0.211 – 3.988)	**0.598**	0.041(0.0 – 5866.199)
FGF19 (positive)	**0.978**	0.986(0.358 – 2.714)	**0.040***	6.555(1.092 – 39.342)

HR: Hazard ratio, C.I: Confidence interval, LL: Lower limit, UL: Upper Limit, #: All variables with p<0.05 was included in the multivariate, *: Statistically significant at p ≤ 0.05

FGFR4 specific targeted drugs are being developed and researched, including reversible (e.g. roblitinib) and irreversible (e.g. fisogatinib) inhibitors. (Weiss et al., 2019, Kim et al., 2019). Most of these agents, however, are only in the early stages of clinical trials and have a long way to go before they can be commonly used in clinical practice.

CONCLUSION

FGF19 is commonly expressed in HCC cases independent from the etiological, background liver or morphological subtypes. FGF19 could be used as an indicator to predict tumour recurrence.

CONFLICTS OF INTEREST

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