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Early Detection and prediction of survival of patients with Hepatocellular Carcinoma Using Transient Elastography

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Abstract

Background:

Detection of early cases of HCC allows for offering curative interventions that may positively impact the survival of patients. Screening depends on the use of ultrasound with or without alpha-fetoprotein. However, some cases might need to be noticed, so alternatives are needed. Transient elastography can be studied as an effective alternative.

Methods:

Patients were divided into groups with liver cirrhosis and HCC and another group without HCC. Demographic criteria and laboratory profiles were compared. Transient elastography was then performed, and values of Controlled Attenuation Parameters (CAP) and liver stiffness were compared between both groups.

Results:

One hundred thirty-three patients with liver cirrhosis and HCC and 133 patients without HCC were studied. Our study showed that patients with HCC had significantly lower steatosis as measured by CAP using transient elastography (p-value < 0.001). However, they had substantially higher fibrosis (p-value < 0.001). A cutoff level of >24.3 Kpa showed a 90.5% sensitivity, 85.7% specificity, 86.4% PPV and 90% NPV for predicting the occurrence of HCC. By using univariate analysis, smoking was a significant predictor for mortality of HCC patients. In contrast, the multivariate analysis showed that gender was an independent

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predictor for mortality in patients with HCC. Liver stiffness measurement was not shown to predict survival in patients with HCC.

Conclusion:

Detection of early HCC is essential to gain the utmost benefit from the available therapeutic options. Transient elastography may provide a tool for diagnosing patients in the early stages. However, it might not be a good predictor of overall survival.

Keywords: Hepatitis C, HCC, Screening, Liver fibrosis, Transient elastography

Introduction

Hepatocellular carcinoma (HCC) is a leading cause of mortality and morbidity globally, specifically in Egypt, where Hepatitis C Virus infection was endemic for decades. This remained a global threat even after the successful introduction of the direct-acting antiviral agent for HCV, and the incidence of HCC was still shown to be there after achieving sustained virological response (SVR) to HCV treatment **(1).**

Surveillance programs are applied to patients with liver cirrhosis to diagnose the occurrence of HCC in early stages, which enables the possibility of providing a curative treatment. Currently, the screening modalities for screening for HCC include ultrasound (US) with or without alpha-fetoprotein (AFP) every six months. However, limitations exist, and false negative results

may be obtained, allowing for missing some cases with HCC, especially those with small lesions. Therefore, other screening modalities are required to aid in catching those with early HCC.

Multiple risk factors exist that can affect the development of HCC. However, the presence of advanced liver fibrosis is the most important and common risk factor in chronic liver disease patients **(2).**

Thus, assessing liver fibrosis can help detect those with a high risk of developing HCC. The most accurate assessment for liver fibrosis can be obtained using a liver biopsy. However, owing to its drawbacks, such as being an invasive technique with potential complications such as bleeding and infection, other non-invasive methods were established, the most accurate to date being transient elastography.

Transient elastography (TE) is a non-invasive diagnostic tool used for evaluating liver fibrosis by measuring the elasticity of the liver parenchyma using ultrasonography (US) ***(3).***

Transient elastography helps diagnose early compensated liver cirrhosis. Accordingly, several liver stiffness-based HCC prediction models using TE have been adopted ***(4).***

In addition, the quantification of liver stiffness was also shown in multiple studies to correlate with the prediction of treatment outcomes and survival in patients with HCC ***(5).***

This study aimed to evaluate the role of Transient Elastography (Fibroscan) in triaging high-risk patients for close follow-up for early prediction of HCC and prediction of their survival.

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Patients and methods

Type of the study:

This is an observational study with a time frame from January 2020 to December 2022, where patients with HCC were recruited from the multidisciplinary HCC clinic at Kasr-Alainy Hospital, Cairo University, Egypt. In addition, we recruited patients with liver cirrhosis without HCC as a control group from the endemic medicine department at Cairo University.

Afterward, patients with HCC were treated according to international guidelines and followed up to detect survival.

HCC was diagnosed according to the criteria in the guidelines of the American Association for the Study of Liver Diseases (AASLD), using computerized tomography (CT) or magnetic resonance imaging (MRI) techniques and alpha-fetoprotein (AFP).

Patients:

1- control group: patients with liver cirrhosis without HCC (133 patients).

2- HCC group: patients with liver cirrhosis with HCC (133 patients).

❖ **Inclusion Criteria:**

1- Chronic hepatitis C virus-related liver cirrhosis patients with or without HCC.

2- Recently diagnosed HCC patients did not receive any previous treatment for HCC.

❖ **Exclusion Criteria:**

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1- Patients with other chronic liver diseases than HCV, e.g., HBV, alcohol-related, autoimmune liver disease, and patients co-infected with HIV.

2- HCC patients who received any previous treatment to HCC.

Methods:

All studied patients were subjected to the following:

Entire history taking and clinical assessment. Baseline laboratory tests were collected in the form of complete blood count, liver function tests, renal functions, Alpha-fetoprotein (AFP) measurement in addition to tumor characteristics according to the dynamic imaging, whether CT or MRI (focal lesion site, size and number, portal vein, and abdominal lymph node assessment).

Transient Elastography (Fibroscan):

The probe was applied on the right hepatic lobe through intercostal spaces on patients lying in the dorsal decubitus position with the right arm in maximal abduction. The tip of the transducer of the probe was placed between the rib bones. The operator, assisted by an ultrasonic time-motion image, located a liver portion away from HCC and free of large vascular structures.

An established diagnosis of cirrhosis was obtained by the following criteria: radiological or transient elastography >12.5 kPa.

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HCC patients were assessed by Eastern Cooperative Oncology Group performance status (PS) **(6)** and managed according to the Barcelona Clinic Liver Cancer (BCLC) guideline **(7)**.

Statistical analysis

All statistical analyses were performed using the SPSS program for Windows (version 20 statistical software; Texas Instruments, IL, USA). Categorical variables are given as numbers and percentages. Continuous data are expressed as the mean and standard deviation or as the Median with the Interquartile range (25% -75%). Comparison between distributions of categorical variables will be performed using the Chi-square (X^2) test. Besides, variables are described as odds ratio (OR) with a 95% confidence interval (95% CI) where appropriate. The data are considered significant t if *p* values were < 0.05 and highly effective if *p* < 0.01. The associations between the gene polymorphisms and HCC stages will be tested using the crosstabs test. The Kaplan-Meier method will be used to calculate the survival rates, and the log-rank test will be used to test the significance of the difference in the patient's survival.

Results

Patients with HCC were significantly older and with male predominance (p-value < 0.001)**.** There was no difference between the studied groups regarding their BMI. Cigarette smoking was significantly more common in patients with HCC (p-value 0.003). We found no significant difference among the studied groups regarding the presence of diabetes mellitus (p-value > 0.05) **(Tab 1)**

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Tab 1. Demographic features of the studied groups.

		Liver cirrhosis group (n = 133)		HCC group (n = 133)		P-value
Sex	**Male**	49	36.8%	105	78.9%	< 0.001
	Female	84	63.2%	28	21.1%	
Age (years) Median (IQR)		47.0 (37.0- 55.0)		62.0 (56.0- 65.0)		< 0.001
Diabetes mellitus		3		2		0.5
Cigarette Smoking		24		39		0.003
BMI (Kg/m2) Median (IQR)		26.80 (24.74- 28.70)		26.40 (24.10- 28.30)		0.329

p≤0.05 is considered statistically significant, p≤0.01 is considered high statistically substantial (HS), X2: Chi-square test, ZMWU: Mann-Whitney U Test.

There was a significant difference between both groups as regards the complete blood count, where hemoglobin level and platelet count were significantly lower in patients with HCC. In contrast, the white cell count was considerably higher. In addition, liver function tests in the form of albumin, bilirubin, and INR were significantly different among both groups, where lower albumin and higher INR levels, as well as more elevated bilirubin and aminotransferase levels, were encountered in patients with HCC. The alpha-fetoprotein level was also significantly higher in HCC patients (p-value <0.001) (**Tab 2).**

*Tab 2. **Laboratory features of the studied groups.***

Variable Median (range)	Liver cirrhosis	HCC	P-value
Hemoglobin (gm/dL)	13.0 (8.5-17.7)	12.9 (1.0-18.4)	**0.02**
WBC (cells/mm3*103)	5.6 (2.3-12.7)	5.8 (2.2-17.0)	**0.498**

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Platelets (/mcL*109)	214.0 (40.0-440.0)	119.50 (36.0-336.0)	**<0.001**
Total Bilirubin (mg/dl)	0.69 (0.10-7.00)	1.20 (0.10-7.00)	**<0.001**
AST (U/L)	37.00 (7.00-565.00)	63.00(7.00-565.00)	**<0.001**
ALT (U/L)	37.00 (4.30-551.00)	56.00 (4.30-551.0)	**<0.001**
Albumin (g/dl)	4.11 (2.00-4.60)	3.30 (2.00-4.60)	**<0.001**
INR	1.00 (0.90-1.67)	1.23 (1.00-2.11)	**<0.001**
AFP (ng/ml)	3.35 (0.50-77.28)	62.50 (2.3-61344.0)	**<0.001**

p≤0.05 is considered statistically significant, and p≤0.01 is considered highly statistically substantial.

Steatosis levels were lower in patients with HCC as measured by CAP using transient elastography (p-value < 0.001). Most patients with HCC (85.7%) had no or mild steatosis (S0 and S1). On the other hand, liver stiffness measurement was significantly different between both groups, showing a higher level in those with HCC (p-value < 0.001) **(Tab 3).**

Tab3. ***Comparisons between studied groups regarding steatosis grade, CAP, and liver stiffness measurements.***

		Liver cirrhosis		HCC		P-value
Steatosis	**S0**	69	51.9%	92	69.2%	**0.001**
grade	**S1**	19	14.3%	22	16.5%	
	S2	27	20.3%	8	6.0%	
	S3	18	13.5%	11	8.3%	
CAP Median (IQR)		234.0 (201.0-267.0)		211.0 (176.0-243.0)		**<0.001**
Liver stiffness Median (IQR)		23.50 (18.5-30.0)		25.0 (20.0-40.0)		**<0.001**

p≤0.05 is considered statistically significant, and p≤0.01 is considered highly statistically substantial.

It was shown that liver stiffness measured by transient elastography can be used to discriminate between patients with liver cirrhosis with and without HCC using a cutoff level of

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>24.3 Kpa with 90.5% sensitivity, 85.7% specificity, 86.4% PPV, and 90% NPV using the ROC curve **(AUC = 0.926 & p-value < 0.001) (Fig 1).**

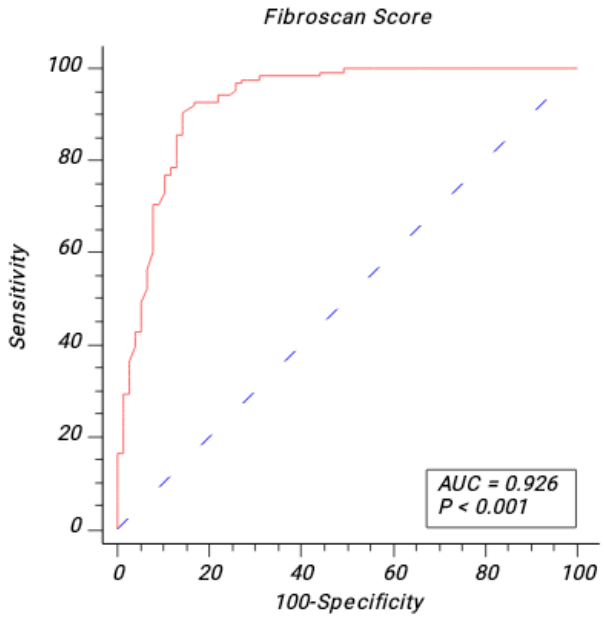

Fig 1. ***ROC curve of liver stiffness in discrimination of liver cirrhosis and HCC.***

The overall survival for patients with HCC was measured from the day of HCC diagnosis till the death of the patient or till the end of the study. The mean overall survival was 29.81 months. Univariate analysis revealed that smoking was a significant predictor of mortality of patients with HCC. In contrast, the multivariate analysis showed that the independent factor affecting mortality was the gender of the patients with HCC **(Tab 4).**

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Tab 4. ***Univariate and multivariate regression analysis of the risk factors for mortality in patients with HCC***

	Univariate analysis				Multivariate analysis			
Parameters	**Odds ratio (OR)**	**95%CI**		**P-value**	**Odds ratio (OR)**	**95%CI**		**P-value**
		Lower limit	**Upper limit**			**Lower limit**	**Upper limit**	
Age	.895	.725	1.104	.299	1.019	.857	1.213	.830
Gender	.495	.137	1.781	.282	43.513	1.098	1723.9	**.044**
Smoking	5.806	1.634	20.63	**.007**	32.161	.860	1202.7	.060
AFP	1.693	.396	7.231	.477	1.000	1.000	1.001	.288
Child score	.389	.053	2.860	.354	1.310	.272	6.316	.736
Treatment response	2.501	2.501	2.501	.264	1.504	.086	26.406	.780
Liver stiffness	.990	.953	1.028	.593	.403	.052	3.090	.382

B: Regression coefficient; S.E.: Standard error, CI: Confidence interval.

Discussion

Hepatocellular carcinoma (HCC) is the sixth and fourth most common cancer worldwide and in Egypt, respectively. Early detection of HCC is paramount since therapeutic approaches are available only for the early stages of HCC. The European and American Associations for the Study of Liver Diseases recommend Ultrasound (US) with or without alpha-fetoprotein (AFP) as a screening modality for HCC. However, the US is less effective in detecting early-stage HCC, with a sensitivity of only 63% **(8)**.

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In addition, Multi-detector CT or dynamic MR imaging is not cost-effective for surveillance in general because of the considerable rate of false-positive findings and the need to use contrast agents to achieve adequate sensitivity **(9)**.

Therefore, establishing alternative screening methods for HCC in patients with liver cirrhosis is required, especially in early cases.

Liver fibrosis is a well-established risk factor for developing HCC, and The HCC incidence rate of cirrhosis is 2–4% per year **(10)**. Thus, liver fibrosis quantification might be a valuable tool for predicting HCC in cirrhotic patients. The gold standard diagnostic method for liver fibrosis is liver biopsy. However, other non-invasive modalities exist due to the invasive nature of liver biopsy. Transient elastography is an approved imaging technique for liver fibrosis assessment, so this study aimed to evaluate the role of transient elastography in predicting the risk of HCC in patients with liver cirrhosis.

This study included 133 patients with liver cirrhosis compared to 133 patients with HCC on top of liver cirrhosis. Most patients with HCC were males, and they were older, which suggests that aging may be related to a higher risk of HCC development. This was stated by Asahina et al., who confirmed that aging and male gender were independent risk factors associated with HCC **(11)**. In addition, a study done by Yi et al. showed that, with advancing age, the effects of alcohol use and HCV on the development of HCC become stronger (**12)**.

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Several observational case–control and cohort studies have reported an increased incidence of HCC in smoker populations, stratified by the severity of smoking **(13)**. In our study, it was shown that there was a statistically significant difference between patients with HCC and patients without HCC as regards tobacco smoking, where those with HCC were more frequent users of Tobacco.

The risk of HCC increases with the advancement in the degree of liver decompensation, and patients with Child scores B and C are shown to have a higher incidence of developing HCC. That was clear in the current study, where synthetic functions of patients with HCC in the form of albumin, bilirubin, and coagulation profile were worse than those without HCC. Also, alpha-fetoprotein level, a recognized glycoprotein marker for HCC, was higher in patients with HCC, with a median value of 62.5 ng/ml. This agrees with several studies that support the utility of AFP as a diagnostic modality for HCC **(14)(15)**. However, other studies raised the debate about the value of AFP in diagnosing HCC. For example, El Serag et al. mentioned that AFP is not always elevated in HCC and showed that its sensitivity and specificity are 79% and 89%, respectively **(16).** Moreover, in small HCC lesions, AFP may show average values **(17).**

Non-alcoholic fatty liver disease (NAFLD) is one of the main etiologies of chronic liver disease and HCC **(18)**. Substantial evidence is present that steatosis is one of multiple risk factors for HCC in patients with chronic HCV infection, even after viral cure by the novel direct-acting antiviral drugs (**19)**. There is ongoing debate about the pathogenic relationship between steatosis and HCC and the potential carcinogenic mechanisms related explicitly to steatosis and steatohepatitis **(20).** In

a study done in 2003, the degree of hepatic steatosis and HCC development were directly proportional **(21).** In addition, it was shown that patients with steatosis had an increased risk of developing HCC by 2.81 times compared to patients who did not have steatosis **(22)**. Obesity, which is also related to the increased prevalence of hepatic steatosis, is also shown to be an independent risk factor for HCC development in patients with chronic HCV **(19).**

On the contrary, a study by Mak et al. in 2021 showed that reduced liver steatosis in patients with chronic hepatitis B infection was inversely proportional to the incidence of HCC. A suggested explanation was that in the terminal stage of steatohepatitis, i.e., the burnt-out phase, no more excess fat is deposited in the liver, which may reflect the lower values of CAP in this population **(22).** Our study agreed with this, as 87.7% of patients with HCC had no or mild steatosis (S0 and S1) compared to 66.1% of patients without HCC. A possible explanation might be that fat deposition might be evaded with advanced fibrosis, tumor infiltration, and lower CAP values.

On the other hand, it was shown that severe fibrosis is directly related to HCC development. A significant correlation was established between the degree of liver stiffness measured by transient elastography in patients with HCC compared to patients with liver cirrhosis who did not develop HCC.

It was shown that liver stiffness measured by transient elastography could be used to discriminate between patients with liver cirrhosis with and without HCC using a cutoff level of

>24.3 kPa with 90.5% sensitivity, 85.7% specificity, 86.4% PPV, and 90% NPV using the ROC curve (AUC = 0.926 & p-value < 0.001)

This observation was evident in many previous studies, which showed that higher fibrosis scores measured by transient elastography increased the risk of HCC development in patients with liver cirrhosis **(23,24,25,26,27).**

Fibrosis and cancer-associated fibroblasts (CAF) can influence liver cancer development by modulating the tumor microenvironment **(28).** Hepatic Stellate Cells play both direct and indirect roles in HCC development. Besides being an essential cell type responsible for fibrosis development, activated HSCs can indirectly support hepatic tumorigenesis by secreting angiogenic factors such as VEGF and angiopoietin 1, and CXC chemokines that can influence tumor vascularization **(29) (30).**

However, different cut-offs were proposed as predictors for HCC development. For example, a study done in 2015 suggested a cut-off of more than 8.5 kPa in patients with chronic hepatitis B and a cut-off of 12 kPa in those with hepatitis C and other etiologies of liver cirrhosis **(31).** A higher cutoff value of 24 kPa was shown in another study for diagnostic prediction of HCC with a sensitivity of 100%, specificity of 83.3%, PPV of 94.5%, NPV of 77.3%, and AUC of 89% **(32).**

In the current study, the overall survival for patients with HCC was measured from the day of the first visit to our multidisciplinary clinic till the death of the patient or till the end of the study. The mean overall survival was 29.81 months. By using univariate analysis, smoking was a

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significant predictor for mortality of HCC patients. In contrast, the multivariate analysis showed that gender was an independent predictor for mortality in patients with HCC.

Liver stiffness measurement by transient elastography failed to be a good predictor for mortality in our study. This contradicted other studies, which showed that higher LSM can be associated with poor overall survival in patients with HCC with different cut-off values. For example, Rekik and his colleagues showed that a TE value ≥ 40 kPa was associated with shorter median overall survival (34 months) compared to a TE value < 40 kPa (59 months, *P* = 0.0008 for the log–rank test) **(33)**.

In conclusion, detecting early HCC is essential to benefit from the available therapeutic options. Liver fibrosis estimation through transient elastography may provide an adequate tool for diagnosing patients in the early stages of the tumor. This might be reflected significantly in the survival and treatment response rates in patients with HCC. Patients with higher LSM are not at an increased risk for HCC-related deaths.

Footnotes.

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Submission declaration:

This work has yet to be published previously and is not under consideration for publication elsewhere. Its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out and, if accepted, will not be published elsewhere, including electronically in the same form, in English or any other language, without the written consent of the copyright holder.

Ethics Approval and Consent to Participate: All procedures followed were by the ethical standards of the responsible committee on human experimentation (Institutional Review Board (IRB)" (N-89-2023) of Cairo University and with the Helsinki Declaration of 1964 and later versions.

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