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EVALUATION OF DIFFERENT ELASTOGRAPHY TECHNIQUES IN PREDICTION OF HEPATOCELLULAR CARCINOMA IN PATIENTS WITH HEPATITIS C RELATED LIVER CIRRHOSIS

By

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ABSTRACT

Background: Although there has been significant improvement in the therapy for HCV achieving a high sustained virological response (SVR). The possibility of developing HCC remains approximately 1% per year after SVR in patients with liver cirrhosis.

Objective: To evaluate the validity of different elastography techniques in prediction of presence of HCC in patients with chronic hepatitis C related liver cirrhosis.

Patients and Methods: This study was a cross-sectional study conducted on Sixty (60) chronic hepatitis C patients (with or without cirrhosis or hepatocellular carcinoma). The studied patients were recruited from Al-Hussein University Hospitals, and National Hepatology & Tropical Medicine Research institute, during the period from 1st of April 2020 to 1st of April 2021. All patients were subjected to full history taking, full physical examination with special emphasis on general examination and abdominal examination, laboratory investigations including CBC, liver function test, renal function test, viral hepatitis markers, serum Alpha-fetoprotein (AFP) level, abdominal ultrasonography, triphasic CT of abdomen and measurement of liver stiffness. Patients were classified into three equal groups: Group I: HCV patients without liver cirrhosis, Group II: HCV patients with liver cirrhosis and no HCC, and Group III: HCV patients with liver cirrhosis and HCC.

Results: FibroScan can be used to discriminate between group A (HCV Without cirrhosis) and group B (HCV with cirrhosis) at a cutoff level of > 11.1, with 100% sensitivity, 100 % specificity, 100% PPV and 100% NPV (AUC = 1.0 and p-value < 0.001). Also, fibroscan can be used to discriminate between group B (HCV with cirrhosis) & group C (HCV with cirrhosis and HCC) at a cutoff level of > 22.6, with 80% sensitivity, 80% specificity, 80% PPV and 80% NPV (AUC = 0.88 & p-value < 0.001). Shear wave elastography (SWE) discriminated between group A (HCV without cirrhosis) and group B (HCV with cirrhosis) at a cutoff level of > 9.6, with 100% sensitivity, 100 % specificity, 100% PPV and 100% NPV (AUC = 1.0 and p-value < 0.001). Also Shear wave elastography (SWE) discriminated between group B (HCV with cirrhosis) and group C (HCV with cirrhosis and HCC) at a cutoff level of > 21.05, with 75% sensitivity, 60% specificity, 65.2% PPV and 70.6% NPV (AUC = 0.68 and p-value = 0.054).

Conclusions: Ultrasound elastographic technology can predict the occurrence of HCC.

Keyword: Elastography, HCC, HCV, Liver cirrhosis.

INTRODUCTION

HCC is the fifth most common cause of malignancy-related death global. Although there has been significant improvement in the therapy for HCV achieving a high sustained virological response (SVR), the possibility of developing HCC remains approximately 1% per year after SVR in patients with liver cirrhosis *(Aleman et al., 2013)*. The gold standard for assessing liver fibrosis and cirrhosis is liver biopsy which is an invasive procedure with rare but potential complications. Instead of liver biopsy, a variety of noninvasive markers have been proposed for assessment of liver fibrosis as risk factor for HCC, i.e. aspartate aminotransferase (AST) to platelet ratio index (APRI), FIB-4, Forns index, Fibro index, FibroTest, Enhanced liver fibrosis, Fibrometer, FIBROSpect II, Hepascore, transient elastography (FibroScan), Shear wave elastography (SWE) and acoustic radiation force impulse imaging (ARFI) *(Schiavon et al., 2014)*.

Development of HCC depends on various risk factors including etiology, race, ethnicity, region, sex, age, presence of diabetes mellitus (DM), and high levels of α-fetoprotein (AFP). However, the presence of advanced liver fibrosis is the most important and common risk factor in chronic liver disease patients *(Bandiera et al., 2016)*. The ultrasound elastography has received widespread attention by adding a new dimension. All liver diseases, focal and diffuse, are associated with changes in the structure of the tissue, with altered liver stiffness (LS), precisely these changes that elastographic techniques can detect and quantify *(Lupsor-Platon et al., 2020)*.

In patients with chronic hepatitis and continuous inflammation of the liver, fibrosis develops during the course of wound healing, with recurrent accumulation of scar tissue and regenerative nodular formation, chronic hepatitis progresses to cirrhosis in untreated patients with chronic hepatitis C infection, which is a leading cause HCC *(Bedossa and poynard et al., 2011)*. The Metavir liver fibrosis stage increases at an annual rate of F0.1 with a concurrent increase in the incidence of HCC *(Shiratori et al., 2013)*.

This present work aimed to evaluate the validity of different elastography techniques in prediction of presence of hepatocellular carcinoma in patients with chronic hepatitis C related liver cirrhosis.

PATIENTS AND METHODS

The current cross-sectional study has been conducted on sixty (60) chronic hepatitis C patients (with or without cirrhosis or hepatocellular carcinoma). The studied patients were recruited from Al-Hussein University Hospitals and National Hepatology & Tropical Medicine Research institute, during the period from 1st of April 2020 to 1st of April 2021. Before starting the study, approval from the Ethics Committee, Faculty of Medicine, Al-Azhar University, Cairo, Egypt, was obtained. Additionally, an informed consent was obtained from all subjects before recruitment in the study. Patients were classified into three equal groups: Group I: HCV patients without

liver cirrhosis, Group II: HCV patients with liver cirrhosis and no HCC, and Group III: HCV patients with liver cirrhosis and HCC. There were exclusion criteria that included patients known to be positive for hepatitis B virus, bilharzial liver cirrhosis, autoimmune hepatitis, primary biliary cirrhosis, obstructive liver disease, alcohol consumption, cardiac cirrhosis, metabolic liver disease and patients not willing to participate in the study.

All patients were subjected to detailed medical history and clinical examination with special emphasis on risk factors of HCV (blood transfusion, previous operations and drug intake), complications of cirrhosis, evidence of stigmata of chronic liver disease as well as abdominal examination. Laboratory investigations were done including CBC, kidney functions tests (blood urea and creatinine), liver function test (ALT, AST, GGT, alkaline phosphatase, total and direct bilirubin), viral hepatitis markers (HCV Ab, HBVs Ag), and serum Alpha-fetoprotein (AFP) level. Radiological investigations in the form of abdominal ultrasonography (Using (Chison 6001) and Triphasic CT of abdomen for all patients for examination of liver echotexture & size, common bile duct diameter, gall bladder stones, portal vein thrombosis, size of spleen, presence or absence of ascites and presence of any masses. Measurement of liver stiffness were done for all patients using Vibration controlled transient elastography (VCTE, FibroScan, Echosens Paris), Shear wave elastography (SWE) using M probe in compliance with the technical recommendations.

Statistical analysis:

Data were analyzed using Statistical Package for the Social Sciences (SPSS) version 24. Quantitative data were expressed as mean± standard deviation (SD) and median Qualitative data were expressed as frequency and percentage. Kruska-wallis test was used to compare groups followed by post-hoc test. Because samples are not usually distributed. P value < 0.05 was considered significant.

RESULTS

Demographic data between studied groups showed that there was no statistically significant difference (p-value > 0.05) as regard sex but there was a statistically significant difference (p-value < 0.001) as regards age **(Table 1)**.

Table (1): Comparisons between studied groups as regard demographic data

Parameters \ Groups		Group A (n = 20)		Group B (n = 20)		Group C (n = 20)		P-value
Sex	Male	17	85%	15	75%	18	90%	0.432
	Female	3	15%	5	25%	2	10%	
Age (years)	Median	25		48.5		60.5		< 0.001
	IQR	23 – 25.8		42.8 – 51		55 - 65		

Liver function tests between studied groups, showed statistically significant differences **(Table 2)**.

Table (2): Comparisons between studied groups as regard liver function tests

Parameters \ Groups		Group A (n = 20)	Group B (n = 20)	Group C (n = 20)	P-value
Total Bilirubin (mg/dl)	Median	0.57	1.55	1.3	< 0.001
	IQR	0.4 - 0.9	0.77 - 1.9	0.62 - 1.77	
Direct Bilirubin (mg/dl)	Median	0.1	0.75	0.35	< 0.001
	IQR	0.1 – 0.2	0.5 – 1.07	0.13 – 0.7	
Albumin (g/dl)	Mean	4.8	3.7	3.5	< 0.001
	±SD	0.3	0.5	0.5	
AST (U/L)	Median	19.5	63.5	45	< 0.001
	IQR	16.5 - 23	44.3 - 89	30.3 - 93.5	
ALT (U/L)	Median	16.5	68.5	45	< 0.001
	IQR	14 - 23.5	43.3 - 91.3	17.7 - 73.5	
INR	Median	0.9	1.25	1.2	< 0.001
	IQR	0.9 - 1	1.13 – 1.3	1.1 – 1.3	
AFP (ng/ml)	Median	5.8	14.8	301	< 0.001
	IQR	4.6 – 7.7	13.1 – 18.7	188 – 1733.5	

AST: aspartate transaminase, ALT: Alanine transaminase.
INR: international normalized ratio, AFP: Alpha fetoprotein.

Complete blood count tests between studied groups, showed statistically significant differences as regards WBCs, Hb and PLTs **(Table 3)**.

Table (3): Comparisons between studied groups as regard CBC

Parameters \ Groups		Group A (n = 20)	Group B (n = 20)	Group C (n = 20)	P-value
Hb (g/dl)	Mean	14.05	12.1	12.1	< 0.001
	±SD	1.6	1.3	1.7	
WBCs (x103/ul)	Median	5.6	4.05	5.05	< 0.001
	IQR	4.6 - 6.6	3.2 - 4.5	3.75 - 6.3	
PLTs (x103/ul)	Median	234	112.5	91	< 0.001
	IQR	196.8 - 282	63.3 - 134	71.5 - 163.5	

FibroScan and SWE tests between studied groups showed statistically significant difference **(Table 4)**.

Table (4): Comparisons between studied groups as regard FibroScan and SWE

		Groups			P-value
		Group A (n = 20)	Group B (n = 20)	Group C (n = 20)	
FibroScan	Median	4.15	20.6	31.9	**< 0.001**
	IQR	3.7 – 4.7	18.7 – 21.8	22.9 – 39.6	
Shear Wave Elastography	Median	4.1	20.6	24.2	**< 0.001**
	IQR	3.6 – 4.6	18.9 – 26.1	21.02 – 34.2	

According to description of Triphasic CT results in group C, as regard to number of lesions, there was 1 lesion in 7 patients (35%), 2 lesions in 3 patients (15%), and 3 lesions in 10 patients (50%). As regard size of lesions, the mean size was 5.27 ± 2.9 with minimum size of 2.5 and maximum size of 14.8 **(Table 5)**.

Table (5): Description of Triphasic CT results in group C

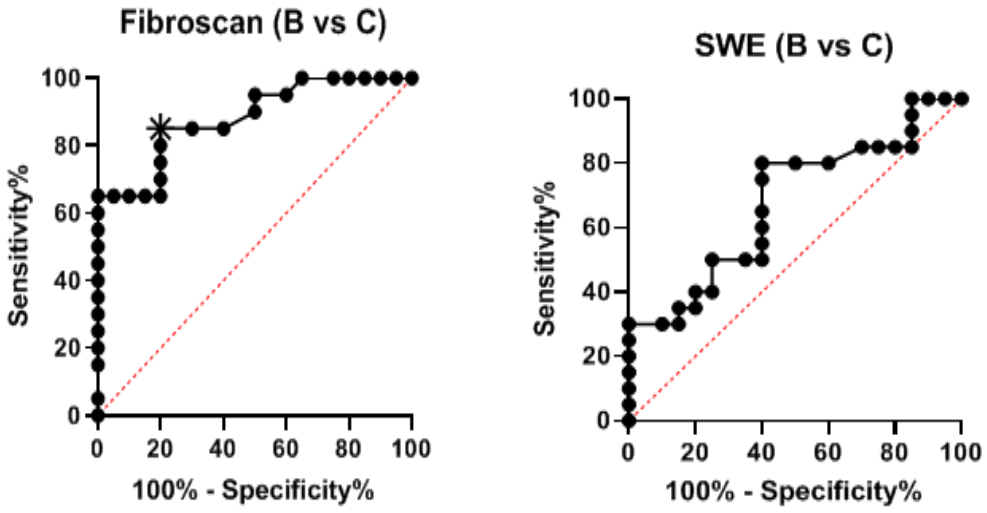
		Group C (n = 20)	
Number of lesions	1 lesion	7	35%
	2 lesions	3	15%
	3 lesions	10	50%
Size of lesions	Mean ±SD	5.27 ± 2.9	
	Min – Max	2.5 - 14.8	

FibroScan can be used to discriminate between group B & group C at a cutoff level of > 22.6, with 80% sensitivity, 80% specificity, 80% PPV and 80% NPV (AUC = 0.88 & p-value < 0.001). SWE can be used to discriminate between group B & group C at a cutoff level of > 21.05, with 75% sensitivity, 60% specificity, 65.2% PPV and 70.6% NPV (AUC = 0.68 & p-value = 0.054) **(Table 6** and **Figure 1)**.

Table (6): Diagnostic performance of FibroScan & SWE in discrimination of group B and Group C

	Cut off	AUC	Sensitivity	Specificity	PPV	NPV	p-value
FibroScan	> 22.6	0.88	80%	80%	80%	80%	< 0.0001
SWE	> 21.05	0.68	75%	60%	65.2%	70.6%	0.054

PPV: positive predictive value; AUC: Area under curve NPV: negative predictive value.



Figure(1): ROC curve

FibroScan was used to discriminate between group A and group B at a cutoff level of > 11.1, with 100% sensitivity, 100% specificity, 100% PPV and 100% NPV (AUC =1.0 and p-value < 0.001). SWE can be used to discriminate between group A and group B at a cutoff level of > 9.6, with 100% sensitivity, 100% specificity, 100% PPV and 100% NPV (AUC = 1.0 & p-value = 0.001) **(Table 7** and **Figure 2)**.

Table (7): Diagnostic performance of FibroScan & SWE in discrimination of group A and Group B

	Cut off	AUC	Sensitivity	Specificity	PPV	NPV	p-value
Fibroscan	> 11.1	1.0	100%	100%	100%	100%	<0.0001
SEW	> 9.6	1.0	100%	100%	100%	100%	<0.0001

PPV: positive predictive value; AUC: Area under curve; NPV: negative predictive value.

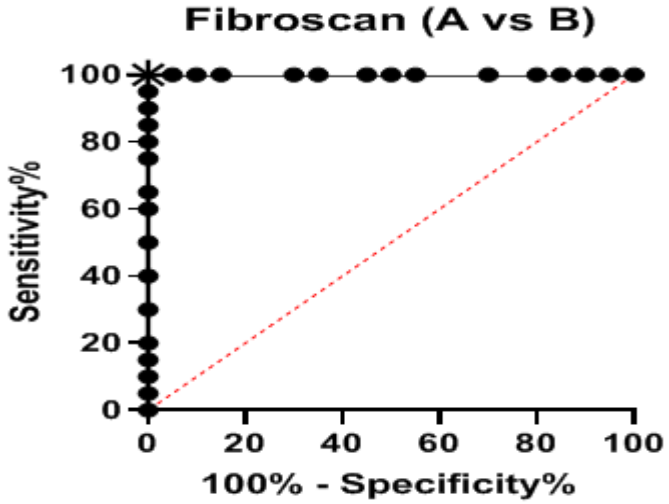


Figure (2): ROC curve

As regard to correlation study between (FibroScan and SWE) and (number of lesions and size) in group C, there were no statistical significant (p-value = 0.897) negative correlations (r = - 0.03) between FibroScan and number of lesions in studied patients. Also, there was no statistical significant (p-value = 0.304) positive correlation (r = 0.24) between FibroScan and size of lesions in studied patients. Also, there was no statistical significant (p-value = 0.713) positive correlation (r = 0.08) between SWE and size of lesions in studied patients. No statistical significant (p-value = 0.967) negative correlation (r = - 0.01) between SWE and size of lesions in studied patients **(Table 8)**.

Table (8): Correlation study between (FibroScan & SWE) and (number of lesions & size) in group C

	(r)	p-Value
FibroScan vs number of lesions	- 0.03	0.897
FibroScan vs size of lesions	0.24	0.304
SWE vs number of lesions	0.08	0.713
SWE vs size of lesions	- 0.01	0.967

(r): Pearson correlation coefficient; was considered non-significant.

Table (9): Post-Hoc test for multiple comparisons between studied groups

		A vs B	A vs C	B vs C
Total bilirubin	LSD	0.86	0.61	0.25
	p-value	< 0.001	0.003	0.198
ALT	LSD	47.3	33	14.3
	p-value	< 0.001	0.001	0.127
AST	LSD	50.6	42.8	7.8
	p-value	< 0.001	< 0.001	0.468
WBCs	LSD	1.8	0.61	1.18
	p-value	0.001	0.250	0.029
PLTs	LSD	129.9	121.6	8.2
	p-value	< 0.001	< 0.001	0.622

DISCUSSION

In the current study we found that, there was no statistically significant difference between studied groups as regard sex, while there was a highly statistically significant difference between studied groups as regard age as the higher age observed in the group C followed by group B and the lower age observed in group A. These results attributed to the higher age individuals more susceptible HCV infection than the young age. Similarly, in *Bandiera et al. (2016),* reported that, development of HCC depends on various risk factors, including etiology, race, ethnicity, region, age, presence of diabetes mellitus (DM), and high levels of α-fetoprotein (AFP). However, the presence of advanced liver fibrosis is the most important and common risk factor in chronic liver disease patients. Also, *Kanwal et al. (2018)* reported that diabetes, and senior ages are independent risk factors for developing HCC. These results agreed with the multivariate analysis study of indicated that, in addition to age, and albumin level, liver stiffness was an independent risk factor associated with HCC incidence *(Masuzaki et al., 2019).*

Our results of serum albumin level cleared that the higher albumin level observed in group (A) followed by group (B) and the lowest level observed in group (C). These results indicated that, the lower albumin level can give indications to higher susceptibility to HCC. These results agreed with those of Yang et al., (2020) from their novel, study where they found that low albumin significantly predicted the development of HCC.

Liver enzymes levels gave indications about degree of hepatitis, our results of liver enzymes indicated that, the AST and ALT levels cleared that, the higher levels observed in group (B) followed by group (C) and the lowest level observed in group (A) and this agreed with *Llovet et al., (2016)* who reported that during the acute phase of acute hepatitis, the liver becomes as stiff as cirrhotic liver, although the stiffness returns to normal as hepatitis improves. Moreover, even among patients with the same fibrosis stage, those with high ALT and AST levels resulting from HCV-induced chronic hepatitis have a high degree of liver stiffness compared with patients with normal ALT and AST levels owing to antiviral treatment or the natural course. This suggests that liver stiffness is affected by the severity of

inflammation as well as by liver fibrosis, leading to an overestimation of liver stiffness when inflammation is present. Therefore, when staging liver fibrosis based on liver stiffness in patients with high ALT and AST levels, it is important to keep in mind that the actual fibrosis stage might be lower.

In a study by *Masuzaki et al: (2019),* investigating the relationship between cirrhosis and the incidence of HCC in patients with hepatitis C, HCC developed in 10 % over the 3-year follow-up period When patients were classified based on liver stiffness at the initial FibroScan examination, patients with a high degree of liver stiffness subsequently had a high incidence of HCC, and this agreed with our results on the comparisons between studied groups as regard FibroScan and SWE, which cleared that, the Fibroscan and Shear Wave Elastography (SWE) showed a higher level in group (C), followed by group (B) and the lowest level was observed in group (A).

Our results cleared that, the FibroScan can be used to discriminate between group A (HCV Without cirrhosis) & group B (HCV with cirrhosis) at a cutoff level of > 11.1, with 100% sensitivity, 100 % specificity, 100%. Also, FibroScan can be used to discriminate between group B (HCV with cirrhosis) & group C (HCV with cirrhosis and HCC) at a cutoff level of > 22.6, with 80% sensitivity, 80% specificity, 80%. According to this result FibroScan can predict HCC in HCV patients at cutoff level > 22.6 with 80% sensitivity, 80% specificity. And our results agreed with a meta-analysis of studies assessing the stage of liver fibrosis using FibroScan, *Friedrich-Rust et al.* found that the cut-off level for a diagnosis of F2 or higher was 7.65 kPa and for a diagnosis of F4, the cut-off level was 13.01 kPa, this demonstrating that fibrosis staging by FibroScan has high accuracy *(Friedrich-Rust et al., 2018).*

This result attributed to the fibroscan machine calculates the degree of liver stiffness (in kPa) by sending acoustic push pulses from the body surface to the liver and following the waves by ultrasound to measure the shear wave speed. The propagation velocity of shear waves correlates with tissue stiffness, with waves traveling faster in harder tissues. FibroScan has several advantages: (1) it is a non-invasive and painless technique, (2) it provides immediate results (within 30 s of measurement), (3) the results are highly reproducible, (4) the measurement area is as wide as 1/500th of the total liver mass (the size of a biopsy specimen is 1/50,000th of the total liver mass), and (5) it can be safely repeated for follow up. On the down side, measurement reproducibility and even the measurement itself can be adversely affected by ascites (push pulses do not travel through fluids) and by thick layers of subcutaneous fat, narrow intercostal spaces, and severe liver atrophy *(Lupsor-Platon et al., 2020).*

Shear wave elastography (SWE) can be used to discriminate between group A (HCV Without cirrhosis) & group B (HCV with cirrhosis) at a cutoff level of > 9.6, with 100% sensitivity, 100 % specificity 100%. Also Shear wave elastography (SWE) can be used to discriminate between group B (HCV with cirrhosis) & group C (HCV with cirrhosis and HCC) at a cutoff level of > 21.05, with 75% sensitivity, 60% specificity

65.2%. According to this result Shear wave elastography (SWE) can predict HCC in HCV patients at cutoff level > 21.05 with 75% sensitivity, 60% specificity. Also, there is no statistically significant correlation between size and number of lesions in patients with HCC in both FibroScan and Shear wave elastography (SWE). Cumulative incidence of HCC development stratified based on liver stiffness measurement (LSM, n = 866) in patients with hepatitis C *(Masuzaki et al., 2019).*

Because liver stiffness is correlated with the stage of liver fibrosis, it is reasonable to assume that liver with a high degree of stiffness indicates advanced liver fibrosis. This strong correlation between liver stiffness and HCC risk also applies to cirrhotic patients, who have a high degree of liver stiffness, suggesting that liver stiffness is a useful clinical indicator to classify patients at high risk of HCC. Our results agreed with those of *Schiavon et al, (2014),* where they reported that, the gold standard for assessing liver fibrosis and cirrhosis is liver biopsy, but it is an invasive procedure with rare but potential complications. Instead of liver biopsy, a variety of noninvasive markers have been proposed for assessment of liver fibrosis as risk factor for HCC, i.e., aspartate aminotransferase (AST) to platelet ratio index (APRI), FIB-4, Forns index, Fibro index, FibroTest, Enhanced liver fibrosis, Fibrometer, FIBROSpect II, Hepascore, transient elastography (FibroScan), Shear wave elastography (SWE) and acoustic radiation force impulse imaging (ARF).

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تقييم التقنيات المختلفة لقياس مرونة الكبد على التنبوء بوجود سرطان الكبد فى مرضى تليف الكبد الناتج عن الالتهاب الفيروسى سى

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خلفية البحث: على الرغم من وجود تحسن كبير في علاج التهاب الكبد الوبائي (سي) وتحقيق إستجابة فيروسية عالية ومستدامة، تظل إمكانية الإصابة بسرطان الكبد حوالي 1٪ سنويًا بعد الشفاء من الالتهاب الفيروسي سي في مرضى تليف الكبد.

الهدف من البحث: تقييم صلاحية تقنيات التصوير الإلستوجرافي المختلفة في التنبوء بوجود سرطان الكبد في المرضى الذين يعانون من تليف الكبد المزمن المرتبط بالتهاب الكبد الوبائي سي.

المرضى وطرق البحث: كانت هذه الدراسة عبارة عن دراسة مقطعية أجريت على ستين (60) مريضًا مزمنًا بالتهاب الكبد C مع أو بدون تليف الكبد أو سرطان الخلايا الكبدية، من مستشفيات الحسين الجامعي والمعهد الوطني لأبحاث الكبد وطب المناطق الحارة، خلال الفترة من 1 أبريل 2020 إلى 1 أبريل 2021. وقد خضع جميع المرضى لأخذ التاريخ الكامل، والفحص البدني الكامل مع التركيز بشكل خاص على الفحص البطني، والفحوصات المخبرية بما في ذلك فحص صورة دم كاملة، وإختبار وظائف الكبد، وإختبار وظائف الكلى، وعلامات التهاب الكبد الفيروسي، ومستوى بروتين ألفا فيتوبروتين، وتصوير البطن بالموجات فوق الصوتية،

والتصوير المقطعي المحوسب ثلاثي الأطوار للبطن وقياس تصلب الكبد. وقد تم تصنيف المرضى إلى ثلاث مجموعات متساوية، المجموعة الأولى شملت مرضي مصابين بفيروس التهاب الكبد الوبائي غير مصابين بتليف الكبد و المجموعة الثانية شملت مرضي مصابين بالتهاب الكبد الوبائي وبتليف الكبد وليس لديهم سرطان الكبد والمجموعة الثالثة شملت مرضي مصابين بفيروس التهاب الكبد سي مع تليف الكبد وسرطان الكبد.

نتائج البحث: يمكن إستخدام جهاز الفيبروسكان للتمييز بين المجموعة أ الالتهاب الفيروسي سي بدون تليف الكبد والمجموعه ب الالتهاب الفيروسي سي مع وجود تليف بالكبد عند مستوى > 11.1، مع حساسية 100٪، وخصوصية 100٪. أيضًا، ويمكن إستخدام جهاز الفيبروسكتن للتمييز بين المجموعة ب الالتهاب الفيروسي سي مع وجود تليف بالكبد والمجموعة سي الالتهاب الفيروسي سي مع وجود تليف الكبد و وجود سرطان بالكبد عند مستوى > 22.6، مع حساسية 80٪، وخصوصية 80٪، ويمكن إستخدام جهاز الشيرويف للتمييز بين المجموعه أ الالتهاب الفيروسي سي بدون تليف الكبد والمجموعة ب الالتهاب الفيروسي سي مع وجود تليف بالكبد عند مستوى > 9.6، مع حساسية 100٪، وخصوصية 100٪، ويمكن أيضًا إستخدام جهاز الشيرويف للتمييز بين المجموعة ب الالتهاب الفيروسي سي مع وجود تليف بالكبد والمجموعة سي الالتهاب الفيروسي سي مع وجود تليف بالكبد و وجود سرطان بالكبد عند مستوى > 21.05، مع حساسية 75٪، وخصوصية 60٪.

الاستنتاج: التطورات الحديثة في تقنية التصوير المرن بالموجات فوق الصوتية يمكن أن تتنبأ بحدوث سرطان الكبد.

الكلمات الدالة: التصوير المرن، سرطان الكبد، التهاب الكبد الوبائي، تليف الكبد.