Efficacy of Real-Time Tissue Elastography (HI-RTE) for the Evaluation of Hepatic Fibrosis in Patients with Chronic Hepatitis C

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Abstract

**Background:** Diagnosis of the stage of liver fibrosis is essential for making a prognosis regarding the development of cirrhosis and hepatocellular carcinoma; and surveillance in patients with chronic HCV. Hitachi Real-time Elastography (HI-RTE) showed promising results as an effective measure for diagnosis of liver fibrosis, regardless of the stage, in patients with chronic viral hepatitis.

**Aim of Study:** We aimed at evaluating the efficacy of (HI-RTE) in staging of liver fibrosis in chronic HCV patients compared to liver biopsy. Results of FIB-4 and APRI scores as non-invasive measures for assessment of liver fibrosis are also discussed in this report.

**Patients and Methods:** A total of 194 chronic HCV patients were recruited and subjected to baseline HCV pre-treatment assessment, including Liver function tests, complete blood picture, prothrombin time, serology for hepatitis B, HCV-RNA, and abdominal ultrasound examination. FIB-4 and APRI scores were calculated. HI-RTE, and finally, ultrasound guided liver biopsy, were performed for every patient.

**Results:** AUROCs of LFI, FIB-4 and APRI were 0.721, 0.774, and 0.744 respectively for detecting significant liver fibrosis (F2), while in predicting advanced liver fibrosis (F3), AUROCs were 0.734, 0.86, and 0.803 respectively, whereas, for detection of liver cirrhosis (F4), AUROCs were 0.841, 0.927, and 0.863 respectively. Cut-off values of LFI for predicting significant fibrosis (F2), advanced fibrosis (F3) and liver cirrhosis (F=4), were 2.67, 2.97 and 3.35 respectively.

**Conclusion:** Liver fibrosis index measured by real time elastography (HI-RTE) performs well and is clinically applicable for evaluating the degree of liver fibrosis in chronic HCV patients.

**Key Words:** HCV – Liver fibrosis – FIB-4 – APRI – Real time elastography.

Introduction

**HEPATITIS C** virus (HCV) is a globally prevalent pathogen and a leading cause of death and morbidity. HCV infection is one of the main causes of chronic liver disease and hepatocellular carcinoma (HCC) worldwide and is the most common indication for liver transplantation (LTx) [1]. In Egypt, hepatitis C is highly endemic where; Egypt DHS of 2015 reported that hepatitis C seroprevalence in the age groups 15-59 years was 10% (compared to 14.7% of the population in the 2008 DHS) [2]. However, recent screening results showed that the HCV seroprevalence among untreated persons was 4.61% [3]. Diagnosis of the stage of liver fibrosis is essential for making a prognosis regarding the development of cirrhosis and hepatocellular carcinoma; and deciding an antiviral therapy and follow-up both during treatment and after cessation of treatment [4]. Liver biopsy was considered for long time as the gold standard to assess liver fibrosis in chronic HCV patients. However, liver biopsy is an invasive procedure, and its accuracy to assess fibrosis has also been questioned concerning sampling errors and intra- and inter-observer variability that may lead to over- or under staging [5]. Also, liver biopsy does not allow dynamic evaluation of liver fibrosis over time [6]. Non-invasive assessment of liver fibrosis has been the focus of research for many years to distinguish between minimal, clinically significant fibrosis and liver cirrhosis includ-
ing serological tests and imaging techniques [7]. Serological biomarkers usually include combination of clinical and laboratory parameters in a specific model. Some of the most popular indices include Fib-4 score, APRI score & modified APRI score [8]. Among imaging techniques, transient elastography (TE) Fibroscan® is widely used as non-invasive method that measures mean liver stiffness [8]. Real-time Tissue Elastography (HI-RTE: “Hitachi Medical Co., Japan”) is a relatively new method for measuring tissue elasticity. It is a simple and non-invasive method for assessing liver fibrosis, which can be performed during a routine liver ultrasound examination without the need for any additional equipment [9]. In patients with chronic hepatitis C, (HI-RTE) allows for non-invasive liver fibrosis assessment that does not vary with the sites tested or by the observer and performs better than serum fibrosis markers [10]. HI-RTE is considered a valuable ultrasound based non-invasive method for assessment of liver fibrosis and has better discrimination power for significant liver fibrosis and early liver cirrhosis than APRI in chronic hepatitis B (CHB) [11].

Patients and Methods

Patient enrollment:

Patients were recruited from among chronic HCV patients attending to Endemic Medicine Department and Viral Hepatitis Center at Faculty of Medicine, Cairo University, and Hepatology clinic of National Hepatology & Tropical Medicine Research Institute (NHTMRI) 2014 and 2015. These patients were evaluated to receive antiviral treatment in the form of Pegylated Interferon/Ribavirin (PegIFN/ RBV) and/or directly acting antiviral agents (DAAS). All participants were subjected to the following:

- Full history taking: Including age, sex, and possible risk factors for viral acquisition.
- Clinical examination: Particular emphasis on manifestations suggestive of chronic liver disease and calculation of Body Mass Index (BMI) in kg/m².
- Routine laboratory investigations: (a) Biochemical liver profile: (Serum bilirubin, ALT, AST, alkaline phosphatase, GGT, and serum albumin). (b) Complete blood picture and PT, PC & INR. (c) Serum creatinine. (d) Viral hepatitis markers (HBsAg, quantitative HCV-RNA assay by PCR). (e) Anti-Schistosomal antibody. (f) Alpha fetoprotein (AFP).
- Conventional ultrasound (US) examination of the liver: Liver parenchymal echopattern is expressed as normal (homogenous), bright or coarse echopattern.
- Calculated scores:
  - APRI score was calculated using Wai’s formula [12]: “(AST/upper limit of normal) / platelet count (10⁹/L) x 100”.
  - FIB-4 score was calculated using Sterling’s formula [13]: “Age (years) x AST (IU/L) / platelet count (10⁹/L) x ALT (IU/L)”.
- Real-time Tissue Elastography (HI RTE): Patients are examined in a supine position with the right arm elevated above the head and are instructed to hold their breath. The examination is performed on the right lobe of the liver through the intercostal space. The RTE equipment displays two images simultaneously; one shows the region of interest (ROI) as a colored area, and the other indicates the conventional B-mode image. We choose an area where the tissue is free from large blood vessels and near the biopsy point. The measurement is fixed to a rectangle 30mm in length and 20mm in breadth located 510mm below the liver’s surface. The color in the ROI is graded from

Study oversight:

This study was conducted in accordance with Good Clinical Practice guidelines laid down in Helsinki Declaration 1975 and was approved by the ethical committee of Faculty of Medicine, Cairo University, Egypt. All patients gave informed consent, including the study procedures, and approved the usage of blood sampling and possible data application in future research.

Study design and assessments:

This is a cross-sectional study conducted on 194 chronic HCV patients who intended to receive antiviral treatment in the form of Pegylated Interferon/Ribavirin (PegIFN/ RBV) and/or directly acting antiviral agents (DAAS). All participants were subjected to the following:

- Full history taking: Including age, sex, and possible risk factors for viral acquisition.
- Clinical examination: Particular emphasis on manifestations suggestive of chronic liver disease and calculation of Body Mass Index (BMI) in kg/m².
- Routine laboratory investigations: (a) Biochemical liver profile: (Serum bilirubin, ALT, AST, alkaline phosphatase, GGT, and serum albumin). (b) Complete blood picture and PT, PC & INR. (c) Serum creatinine. (d) Viral hepatitis markers (HBsAg, quantitative HCV-RNA assay by PCR). (e) Anti-Schistosomal antibody. (f) Alpha fetoprotein (AFP).
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blue (representing hard areas) to red (representing soft areas). The scale ranged from red for components with the most significant strain (i.e., the softest components) to blue for those with no strain (i.e., the most hardened components). Green indicates average strain in the ROI, and therefore intact liver tissue displayed as a diffuse homogeneous green pattern. An appearance of unevenness in the color pattern is considered to reflect a change in the liver stiffness. For quantification, all pixel data in the colored image are transferred into a histogram and binary image \[14\]. In this study, we used a new generation RTE technique that was developed by Hitachi Medical Systems. The technique utilizes RTE quantitative analysis software, relying on the patient’s cardiovascular pulsation to produce compression. A total of 12 quantitative parameters were calculated automatically using the updated software integrated into the HI VISION Avius ultrasound scanner \[15\].

- Liver biopsy: Percutaneous liver biopsies were performed under ultrasound guidance by a specialist, using 18-G disposable needles. The biopsy specimens were fixed with formalin and stained with Hematoxylin and Eosin. All of the liver biopsies were evaluated by expert pathologists, who were blinded to the patients’ clinical histories. The histologic staging of liver fibrosis was a combinational assessment of the amount of fibrosis and architectural disorganization using the METAVIR semiquantitative scoring system. METAVIR is demonstrating different stages of liver fibrosis as follows: F0: No fibrosis; F1: Portal fibrosis without septa; F2: Portal fibrosis with rare septa; F3: Numerous septa without cirrhosis; F4: Cirrhosis and different grades of necroinflammatory changes activity as follows: A0: No histological activity; A1: Minimal activity; A2: Moderate activity; A3: Severe activity \[16\].

Statistical analysis:
Analysis of data was performed using SPSS 21 (Statistical Package for Scientific Studies) for Windows. Description of quantitative variables was in the form of mean ± Standard Deviation (SD), Median, 25th and 75th percentiles. Description of qualitative variables was in the form of numbers (N) and percents (%). Data was explored for normality. Results indicated that data was normally distributed so parametric tests were used for comparisons. Comparison between quantitative variables was carried out by student t-test of two independent samples. Repeated measures Analysis of Variance (ANOVA) test was used instead of t-test when comparing between more than two groups of independent variables. Results were expressed in the form of p-values. Receiver Operator Characteristic (ROC) curves were constructed to assess the reliability of the noninvasive markers of liver fibrosis and to determine an appropriate score in predicting stage of liver fibrosis that gives optimal sensitivity and specificity. Area under the curve (AUC) was considered reliable if more than 65%. The significance of the results was assessed in the form of p-value that was differentiated into: Non-significant when p-value >0.05, Significant when p-value 0.05, highly significant when p value 0.01.

Results
This study was conducted on 194 chronic HCV selected patients. These patients intended to receive antiviral treatment for HCV in the form of Pegylated Interferon/Ribavirin (PegIFN/ RBV) and/or directly acting antiviral agents (DAAS). They were selected from the Endemic Medicine Department at Faculty of Medicine, Cairo University, and Hepatology clinics of National Hepatology & Tropical Medicine Research Institute from between 2014 and 2015 according to the criteria mentioned above.

The mean age of the studied patients was 42.23 ±10.90 years and the mean BMI was 27.81±4.70. As for gender distribution of the patients, 119 (61.3%) were males and 75 (38.7%) were females.

As shown in Table (1), there was a significant statistical difference among liver fibrosis stages regarding serum albumin and platelet count where lower levels are associated with higher liver fibrosis stage (p-value 0.01). This was in contrast to serum total bilirubin, AST, ALT (p-value 0.01), and alkaline phosphatase (ALP) (p-value 0.05), where higher levels are associated with a more advanced stage of liver fibrosis. On the other hand, direct bilirubin and GGT showed no significant association with hepatic fibrosis (p-value >0.05).

As shown in Table (2) and Fig. (1), constructing the ROC curve of the used noninvasive methods in our study revealed comparable results regarding the diagnostic performance of liver fibrosis index (LFI) by “HI-RTE”, FIB4 and APRI for detection of significant liver fibrosis (F 2), with AUROCs of 0.721, 0.774, and 0.744 respectively. The optimal cut-off value of liver fibrosis index (LFI) for predicting significant fibrosis (F 2) was 2.67 with a sensitivity of 76.7% and specificity of 61%. LFI exhibited lower diagnostic performance than FIB4 and APRI in detecting significant fibrosis.
As shown in Table (3) and Fig. (2), analysis of the ROC curve of non-invasive methods for detection of advanced liver fibrosis (F3) revealed comparable diagnostic performance levels for LFI by (HI-RTE), FIB4 and APRI with AUROCs of 0.734, 0.86, and 0.803 respectively. Cut-off value of LFI for detecting advanced fibrosis (F3) was 2.97 with a sensitivity of 63.4% and specificity of 71%. Yet, Liver fibrosis index by (HIRTE) showed lower diagnostic accuracy than FIB4, and APRI in detecting advanced fibrosis (F3).

As shown in Table (4) and Fig. (3), on constructing ROC curve of non-invasive methods for detection of liver cirrhosis (F4), it revealed comparable diagnostic performance levels for LFI by (HI-RTE), FIB4 and APRI with AUROCs of 0.841, 0.927, and 0.863 respectively. Liver fibrosis index (LFI) by (HI-RTE) showed a lower diagnostic performance level than FIB4 and APRI. The optimal cut-off value of LFI for the diagnosis of liver cirrhosis (F4) was (3.35) with a sensitivity of 88.9% and specificity of 76.8%.
Table (3): Indices of non-invasive methods for detection of advanced liver fibrosis (F3).

<table>
<thead>
<tr>
<th>Test Result Variable(s)</th>
<th>AUC</th>
<th>Std. Error</th>
<th>Asymptotic 95% Confidence Intervals</th>
<th>Best Cut-off</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;RTE&quot; Liver fibrosis index (LFI)</td>
<td>0.734</td>
<td>0.046</td>
<td>0.644 - 0.825</td>
<td>2.97</td>
<td>63.4%</td>
<td>71%</td>
<td>&lt;0.001 **</td>
</tr>
<tr>
<td>Fib4</td>
<td>0.860</td>
<td>0.034</td>
<td>0.795 - 0.926</td>
<td>1.84</td>
<td>70.7%</td>
<td>85.5%</td>
<td>&lt;0.001 **</td>
</tr>
<tr>
<td>APRI</td>
<td>0.803</td>
<td>0.040</td>
<td>0.724 - 0.882</td>
<td>0.66</td>
<td>78%</td>
<td>69%</td>
<td>&lt;0.001 **</td>
</tr>
</tbody>
</table>

*p<0.05 (significant)  **p<0.01 (Highly significant).
AUC : Area under the curve.
Std error : Standard error.
LFI : Liver fibrosis index.
Fib4 : Fibrosis-4 score.
APRI : AST/platelet ratio index.

Fig. (2): ROC Curve of non-invasive methods for the detection of advanced liver fibrosis (F3).

Table (4): Indices of non-invasive methods for detection of liver cirrhosis (F4).

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<tr>
<td>&quot;RTE&quot; Liver fibrosis index (LFI)</td>
<td>0.841</td>
<td>0.054</td>
<td>0.734 - 0.947</td>
<td>3.35</td>
<td>88.9%</td>
<td>76.8%</td>
<td>&lt;0.001 **</td>
</tr>
<tr>
<td>Fib4</td>
<td>0.927</td>
<td>0.029</td>
<td>0.870 - 0.985</td>
<td>3.20</td>
<td>77.8%</td>
<td>93.8%</td>
<td>&lt;0.001 **</td>
</tr>
<tr>
<td>APRI</td>
<td>0.863</td>
<td>0.051</td>
<td>0.763 - 0.964</td>
<td>1.59</td>
<td>77.8%</td>
<td>88.1%</td>
<td>&lt;0.001 **</td>
</tr>
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*p<0.05 (significant).  **p<0.01 (Highly significant).
AUC : Area under the curve.
Std error : Standard error.
LFI : Liver fibrosis index.
Fib4 : Fibrosis-4 score.
APRI : AST/platelet ratio index.

Fig. (3): ROC Curve of non-invasive methods for the detection of liver cirrhosis (F4).
Discussion

Routinely used laboratory parameters as markers of liver fibrosis:

This study shows significant relation between liver fibrosis and several routinely used laboratory parameters, especially serum AST, bilirubin, albumin level, and platelet count.

There was a significant relation between platelet count and severity of liver fibrosis, where lower platelet count is with higher grades of liver fibrosis. Platelet count decreased with significant liver fibrosis (F2), advanced fibrosis (F3), and liver cirrhosis (F4). This is consistent with Kandemir et al., [17] and Hung-Wei et al., [18].

Our results support the role of serum AST level as a good predictive variable for detection of liver fibrosis, where higher levels are associated with more advanced stages of liver fibrosis in CHC patients, which is in concordance with Drees et al., [19]. Previous studies also show that low AST values significantly correlate with less severe histological parameters and extent of liver fibrosis, especially when combined with platelet count [20], age, total cholesterol level, insulin resistance, and past alcohol intake [21].

The study results showed that serum albumin is a good predictive variable in detecting significant liver fibrosis (F2) and distinguishing patients with advanced liver fibrosis (F3-F4) and liver cirrhosis (F4) from those with lower grades of liver fibrosis. This comes in agreement with Nassef et al., [22] and Forns et al., [23], which showed a gradual fall in serum albumin in chronic hepatitis with cirrhosis progression.

Previous studies showed that serum bilirubin level alone correlates poorly to the stage of fibrosis as it increases late in the disease: its diagnostic value increases when it is used as a part of an index to discriminate severe fibrosis. Total bilirubin >1mg/dl showed 86% sensitivity and 50% specificity for advanced fibrosis (METAVIR F3-F4) [24]. Koda et al., [25] showed that serum bilirubin concentrations increased along with the increased severity of fibrosis among CHC patients, and this is in concordance with our results. Also, total serum bilirubin and direct bilirubin in this study showed significantly higher levels in association with liver cirrhosis (F4) as compared to non-cirrhotic patients (F0-F3), and this comes in agreement with Nassef et al., [22].

Detection of significant liver fibrosis:

Liver fibrosis index (LFI) by (HI-RTE) showed significantly high diagnostic accuracy for differentiating significant fibrosis (F2-F4 versus F0-F1) with the area under the receiver operating characteristic (AUROC) curve of (0.721) with sensitivity 76.7% and specificity 61% at the cut-off value 2.67. This agrees with results obtained by Wu et al., [26].

This study demonstrated comparable performance levels among the used methods in detecting significant fibrosis (F2). LFI by (RTE) exhibited lower diagnostic performance with (AUROC) 0.721 versus 0.774 and 0.744 for FIB4 and APRI respectively. This is correspondent with the study of Ferraioli et al., [27], which revealed that, on distinguishing significant from non-significant fibrosis in chronic hepatitis C patients, RTE fibrosis index (LFI) was inferior to (LSM) by Fibroscan® and APRI. It is also consistent with the studies of Bonnard et al., [28], and Alboraie et al., [29]. That found that FIB4 was superior to APRI in predicting significant fibrosis (F2).

Predicting advanced liver fibrosis:

This study results showed that (LFI) exhibited a high diagnostic performance level for predicting advanced fibrosis (F3) at a cut-off value of 2.97 with a sensitivity of 63.4% and specificity of 71%. This matches with results of the studies conducted by Yada et al., [30], and Marques et al., [31] that revealed that (LFI) calculated by RTE has a very good diagnostic performance to predict advanced fibrosis (F3-F4 versus F0-F2) in CHC patients at a cut-off value of 2.38 with remarkable sensitivity and NPV.

By comparing the different used non-invasive methods for diagnosing advanced fibrosis (F3) in studied patients, it revealed that diagnostic performance level of (LFI) by (RTE), FIB4, and APRI are comparable. LFI by (RTE) showed lower diagnostic performance with (AUROC) 0.734 versus 0.860 and 0.803 for FIB4, and APRI respectively. This is consistent with Ferraioli et al., [27], who concluded that APRI offered higher diagnostic performance in assessing severe fibrosis (F3) (AUROC=0.89) than LFI by RTE (AUROC=0.80). It is also consistent with the study of Alboraie et al., [29] and Yosry et al., [32], who found that FIB4 was more efficient predictor of advanced fibrosis than APRI.

These results differ from the results of Yada et al., [30] and Tamaki et al., [33], which revealed that in patients with CHC, RTE liver fibrosis index was a very accurate tool in predicting advanced fibrosis (F3) and outperformed blood markers (FIB-4 and APRI) in diagnostic capacity.
Detecting liver cirrhosis:

This study revealed that LFI by (HI-RTE) has significant high diagnostic accuracy in differentiating liver cirrhosis (F4 versus F0-F3) at the cut-off value of 3.35 with 88.9% sensitivity and 76.8% specificity. This agrees with Ge et al., [15], who reported that (LFI) at a cut-off value of 3.25 for diagnosing cirrhosis stage resulted in a sensitivity of 100%, a specificity of 88.9%, and an accuracy value of 90.8%.

By comparing the different non-invasive methods for detecting liver cirrhosis (F4), we found that all of the used methods exhibited comparable diagnostic performance levels with AUROCs of 0.841 for (LFI) by (RTE) versus 0.927 and 0.863 for FIB4 and APRI respectively. This is compatible with the study of Ferraioli et al., [27], which revealed that LFI by (RTE) exhibited a lower diagnostic performance level (AUROC 0.80) in predicting liver cirrhosis (F4) than that of APRI (AUROC 0.84) and this is matching with our study results. Our study results are also consistent with the study of Alborai et al., [29], who found that FIB4 was more efficient measure for diagnosing liver cirrhosis than APRI.

Conclusion:

Liver fibrosis index (LFI) measured by real-time elastography (HI-RTE) performs well and is clinically applicable for detecting liver fibrosis. LFI by HI-RTE is nearly as effective as FIB4, and APRI. They all show high reliability as noninvasive methods in predicting and staging liver fibrosis in CHC patients with comparable diagnostic performance levels. Nevertheless, LFI by HI-RTE offered lower diagnostic performance in assessing liver fibrosis than FIB4 and APRI. However, we believe that further studies may increase the predictive value of real-time elastography in assessing liver fibrosis.

References


342 Efficacy of HI-RTE for Evaluation of Liver Fibrosis in CHC


دراسة فعالية قياس مطاطية الكبد (جهاز هيتاشي) كأداة لتقييم درجة تكوّن النسيج الليفي في الكبد في مرضى الإلتهاب الكبد الفيروسي (ج) المزمن

يعتبر الإلتهاب الكبد الفيروسي (ج) من الأمراض المزمنة في مصر وسبباً للأمراض والوفيات، ظلّت الميزة الكبدية لفترة طويلة هي المعيار الذي تم تقدير درجة تكوّن النسيج الليفي في الكبد، ولكنها طريقة تداخلية ومتكلفة وقد يحدث عنها مضاعفات جانبية للمريض علاوة على وجود فروق فردية في فحصها وتشخيصها. إذاً نحن نركز البحث العلمي مؤخراً لإيجاد طرق غير تداخلية لتقييم درجة تكوّن النسيج الليفي بالكبد.

تهدف الدراسة تحديد مدى فعالية جهاز هيتاشي لقياس القياس الوعائي لفترة الكبد (HI-RTE) في تقدير درجة تكوّن النسيج الليفي في الكبد في مرضى الإلتهاب الكبد الفيروسي (ج) المزمن مقارنة بالعينة الكبدية.

أجريت الدراسة على (194) مريضاً مصاباً بالإلتهاب الكبد الفيروسي (ج) المزمن، وقد تم عمل فحص الكبد بتجميع جهاز هيتاشي (HI-RTE) لقياس الوعائي لفترة الكبد (HI-RTE) بالإضافة إلى فحوصات تقييم المريض قبل بدء علاج فيروس (ج)، وفي النهاية تم أخذ المئاتية الكبدية، وذلك للمقارنة بين جهاز هيتاشي (HI-RTE) لقياس الوعائي لفترة الكبد (HI-RTE) وطرق أخرى لتقييم درجة تكوّن النسيج الليفي في الكبد.

وقد أثبتت الدراسة على انبعاث (APRI) و (FIB4) في بالآلات يمكن الاستخدام عليها للتنبؤ بدرجة تكوّن النسيج الليفي الكبدى في مرضى الإلتهاب الكبد الفيروسي (ج) المزمن.

أثبتت الدراسة أن مؤشر التهاب الكبد المحتسب عن طريق جهاز هيتاشي لقياس الوعائي لفترة الكبد (HI-RTE) لديه إمكانية بشكل فعال في تحديد درجات تكوّن النسيج الليفي بالكبد، وذلك مقارنة بالعينة الكبدية، وتستن من خلال هذه الدراسة أن استخدام هذا الجهاز جيد وقابل للتطبيق سريعاً. كما أثبتت الدراسة أن فعالية تقييم درجة تكوّن النسيج الليفي في الكبد في مرضى المصابين بالإلتهاب الكبد الفيروسي (ج) المزمن.