

Transient Elastography can Predict the Risk of Hepatocellular Carcinoma in Egyptian Patients with Chronic Hepatitis C

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ABSTRACT

AIM: HCC is one of the most serious causes of cancer mortality in Egypt due to the burden of HCV, liver stiffness measured by elastography may be useful in demarcating patients at risk for HCC development. This study is aim to verify whether FibroScan could be used as a tool for identifying HCV positive patients who are at high risk of developing HCC.

METHODS: The study included 150 patients with proven chronic hepatitis C (CHC) without HCC (group I), and 100 patients with CHC and HCC (group II). HCV patients were diagnosed by HCV Ab; serum HCV RNA and US liver biopsy. HCC was diagnosed based on alpha-feto-protein, triphasic spiral CT with double contrast and US guided biopsy when needed. Liver stiffness measurement by transient elastography (FibroScan®- Echosens) was carried out for all the patients included in the study. Multivariate logistic regression was applied to assess the association with HCC presence. We computed ROC curve concerning the prediction of HCC. We calculated stratum-specific likelihood ratios (SSLR) for the two groups.

RESULTS: Multivariate analysis showed a positive correlation between the liver stiffness & the presence of HCC & the number of focal lesions ($P < 0.001$). SSLR for HCC presence by liver stiffness was 0.0128 in < 10 kPa, 0.8189 in 10.1 to 15 kPa, 4.285 in 15.1 to 25 kPa, and 15 in > 25 kPa. When using ROC curve, stiffness showed high significant sensitivity 97% and specificity 84% at the level of > 12 kPa. The positive predictive value was 85% and negative predictive value was 96.6%. Univariate analyses showed that the risk of HCC increased in accordance with SSLR; older age, male gender, clinical

cirrhosis, lower serum albumin level, higher total bilirubin level, higher ALT & AST levels & lower prothrombin $p < 0.001$. It also showed high significant negative correlation with PC% and albumin. **CONCLUSION:** Liver stiffness measured by FibroScan® could be a useful predictor of HCC development in patients with CHC. Patients expressing fibroscan score > 25 kPa. and SSLR > 4.1 are in need for closer follow up.

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Key words: Chronic hepatitis C; HCC; Fibroscan; SSLR; Stiffness

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common primary hepatic malignancy worldwide^[1] while being the first cause of cancer mortality in Egypt^[2]. The population of Egypt has a heavy burden with HCV (14.7%), so it has been projected that the number of cases of HCC will continue to increase for the next three decades^[3]. There is a doubling in the incidence rate in the past 10 years^[2]. It is estimated that the problem of HCC will increase until it reaches its peak in the year 2018^[4].

In approximately 20% of patients infected with HCV, this process will lead to cirrhosis within 20 years of infection. Among cirrhotic patients, 1-4% per year will develop of HCC^[5]. Soa screening program for HCC detection is recommended to all patients with cirrhotic liver^[6].

Ultrasound elastography, commercially known as FibroScan®, uses a modified ultrasound probe to measure the velocity of a shear wave created by a vibratory source^[7]. Ultrasound elastography can be performed in approximately 95% of patients. This technique has been evaluated most consistently in patients with chronic HCV disease^[8]. FibroScan has a positive correlation with the fibrosis, according to the METAVIR scoring system, independently of the liver disease etiology^[9]. The degree of liver fibrosis is the strongest indicator of risk for hepatocellular carcinoma (HCC) development,

that's why liver stiffness measured by transient elastography is useful in demarcating patients at a high risk for HCC, who require frequent check-up by imaging examinations^[10]. So we aimed to verify whether ultrasound elastography (FibroScan®), could be used as a tool for identifying Hepatitis C virus positive patients who are at high risk of developing HCC.

MATERIALS AND METHODS

Patients

This study was conducted on 250 Chronic HCV patients admitted to the National Hepatology & Tropical Medicine Research Institute (NHTRMI) in the period between January 2010 and August 2010. An informed consent was obtained from all participants and the study was approved by the ethical committee. Patients with other liver diseases were excluded from the start.

Inclusion criteria: Adult chronic naive HCV patients of both sexes who were diagnosed by serological, virological and histological evidence of HCV-related chronic liver disease in absence of other causes of liver disease.

Naive adult HCC patients on top of chronic HCV as proved by triphasic CT or by Histopathological examination with no history of any ablation trial.

Exclusion criteria: Patients with ascites, DM, Obesity, previous anti-viral therapy, HIV; evidence of liver disease other than hepatitis C (e.g. autoimmune hepatitis, metabolic disorders, HBV, or Schistosomiasis). The studied patients were classified into: Group I: 150 patients with chronic HCV infection and no HCC; Group II: 100 patients with chronic HCV infection with HCC.

Methodology

Routine work up: All patients included were subjected to full medical history and clinical evaluation including Child's scoring. Routine laboratory tests were done including (blood sugar- CBC- alpha-fetoprotein- liver and kidney biochemical profiles). HCV patients were diagnosed by HCV Ab; HCV RNA and US liver biopsy.

Abdominal ultrasonography was performed to all patients. Triphasic spiral CT scan with double contrast was done to all patients in the HCC group (GII) for the diagnosis of hepatic focal lesions with specific features of HCC. In six patients of them the focal lesions did not show the characteristic enhancement pattern for HCC so biopsy was done using trucut needles guided by abdominal U/S.

Specific examination: Transient elastography: Liver stiffness was measured for the 250 patients using the transient elastography machine "Fibroscan" manufactured by Echosens.

Results were expressed in kiloPascals (kPa) and 10 validated measurements were recorded for each patient. Liver stiffness values ranged from 2.5 to 75 kPa according to the case; as it is differ among cases with only chronic hepatitis C, cirrhosis or HCC. The results are immediately recorded by the machine^[11] The validity of TE results depends on two important parameters: (1) the interquartile range (IQR), which reflects the variability of the validated measures, and should not exceed 30% of the median value; (2) the success rate (the ratio of the number of successful measurements to the total number of acquisitions) should be at least 60%; these two items limit the differences in the measurements as the technique is operator dependant^[12].

Stratum-specific likelihood ratio: Peirce & Cornell^[14]. Defined SSLR as the ratio of 2 probabilities, the probability of a test result within a given range, or stratum, when the disease is present, divided by the probability of the same test result when the disease is absent. By using SSLR, we can estimate the posttest probability for any given test result. Beck in (1986)^[13], calculated SSLR as the proportion of diseased

subjects with a test result in a given range divided by the proportion of non diseased subjects with a test result in the same given range.

Statistical analysis

Patients' data were tabulated and processed using SPSS (17.0) statistical package for Windows XP. Comparison between the two groups was done by T-student test and Chi-square test for quantitative and qualitative variables respectively. Spearman Correlation coefficient test was used to rank different variables against each other's positively or inversely. ROC (receiver operator characteristic curve) was used to find out the best cut off value, sensitivity, specificity and area under the curve.

RESULTS

The study included 150 patients with CHC and post hepatic cirrhosis G I and 100 patients with HCC developed on top of CHC G II. There was significant age difference towards the development of HCC compared to chronic HCV and HCV-cirrhosis, *p* value <0.001. The male: female ratio was (2:1) in both groups showing no statistical difference (*p*>0.05). Also the different laboratory parameters and the patients' Child classification were presented. There was a significant difference between both groups regarding Alfa fetoprotein (*p*< 0.024). Regarding sensitivity and specificity of serum AFP, HCC was diagnosed at level of 200 µg/dL^[15]. This revealed a very low sensitivity of AFP as a diagnostic test for HCC (22%), with high specificity (100%). The positive predictive value was 100%, while the negative predictive

Table 1 Patients epidemiological and laboratory characteristics.

| Variables | Non HCC (GI) N=150 Mean±SD | HCC (GII) N=100 Mean±SD | <i>P</i> value |
|---|----------------------------------|-------------------------------|----------------|
| Age, year | 37.56 ±9.75 | 53.21± 7.80 | <0.01 |
| Male/female <i>n</i> | 97/53 | 63/37 | >0.05 |
| Child scoring A/B/C | 119/31/0 | 47/38/15 | ----- |
| AST (0-37 U/L) | 53.66±43.30 | 61.90±36.37 | <0.04 |
| ALT (0-41 U/L) | 54.56±32.25 | 63.53±41.71 | <0.77 |
| ALP (40-104 U/L) | 66.47 ±24.44 | 245.95 ±147.74 | <0.002 |
| P.C% | 88.74±11.24% | 81.45±16.77% | <0.001 |
| Albumin (3.5-5 g/dL) | 4.22±0.49 | 3.53±3.21 | <0.037 |
| Total bilirubin (up to 1mg/dL) | 0.77± 0.28 | 1.63±1.49 | <0.001 |
| Creatinine (up to 1.2 mg/dL)mg/dL | 0.94±0.29 | 1.01±0.32 | <0.22 |
| AFP ng/dL | 16.11±7.99 | 985.57±233 | <0.024 |
| WBC (×10 ³ cells mm ³) | 5250.90±1779.66 | 3758.80±1128.36 | <0.001 |
| HB (g/dL) | 12.04±1.30 | 10.97±1.36 | <0.001 |
| PLT (×10 ³ /µL) | 267.06±100.15 | 165.95±75.48 | <0.001 |

p>0.05 (insignificant); *p*<0.05 (significant); *p*<0.01 (Highly significant).

value was 56.18%. All the previous data was presented in table 1.

The characteristics of the FHL of GII were shown in figure 1. The CT pattern of HCC in triphasic CT scan: 94 lesions (94%) showed typical enhancement features of HCC (typical special pattern of arterial uptake followed by venous washout in the delayed portal/venous phase), while 6 lesions (6%) showed atypical enhancement pattern on different CT scan phases. So US guided liver biopsies with histopathological examination were done for these lesions that showed atypical enhancement pattern in triphasic; 3 lesions were grade I, while the others 3 lesions were grade II.

Fibroscan results

Stiffness range was 2.90-39.80 kpa among CHC (GI) patients with a mean of 8.95±7.27 kpa, while among HCC (GII) patients; its range was 8.40-75.00 kpa with a mean of 31.10±16.05 kpa (Figure 1).

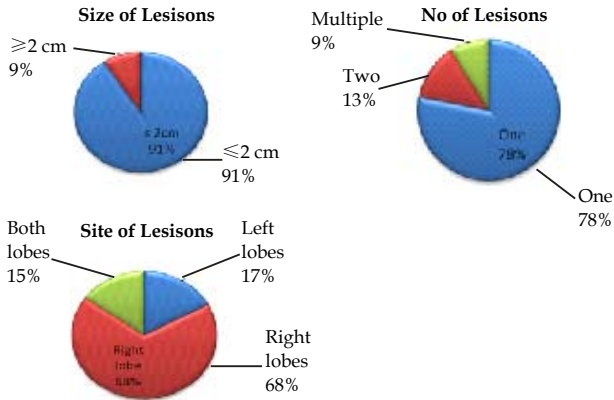


Figure 1 Ultrasound features of the hepatic focal lesions of the studied group.

On comparison between both groups, there was a highly significant difference between both groups regarding liver stiffness ($p < 0.01$). In the NonHCC group (G I), 61 (40.6%) patients were F1, 21 (14%) were F2, 31 (20.7%) were F3, and 37 (24.7%) were F4. While in the HCC group (G II), no patients (0%) were F1, only one (1%) patient was F2, 2 (2%) were F3, and 97 (97%) were F4.

Since we were aiming to use LSM as an indicator of risk of HCC, rather than a diagnostic tool, the values of the LSM in both groups were distributed in different ranges of values (Strata) and the likelihood ratio of incidence of HCC was calculated for each strata.

We arbitrarily distributed the values of liver stiffness into four strata and calculated the SSLR (Stratum-specific Likelihood Ratio). SSLR for HCC presence by liver stiffness was 0.0192 in < 10 kPa, 0.5625 in 10.1 to 15 kPa, 1.6071 in 15.1 to 25 kPa, and 4.0909 in > 25 kPa. There were 22 chronic HCV patients showed SSLR of 4.0909 in > 25 kPa. When using ROC curve, stiffness showed high significant sensitivity 97% and specificity 84% at the level of > 12 kPa. The positive predictive value

Table 2 Stratum-specific Likelihood Ratio Analysis of Liver Stiffness.

| Strata | Non HCC (G I) (n=150) | HCC (G II) (n=100) | SSLR |
|-------------|--------------------------|-----------------------|--------|
| < 10 kpa | 76 | 1 | 0.0192 |
| 10.1-15 kpa | 24 | 9 | 0.5625 |
| 15.1-25 kpa | 28 | 30 | 1.6071 |
| > 25 kpa | 22 | 60 | 4.0909 |

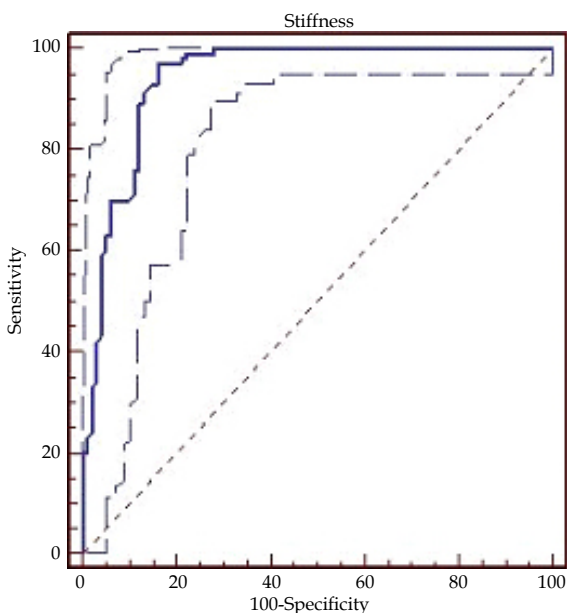


Figure 2 ROC curve comparing stiffness [Area under the ROC curve (AUC)=0.941; $z=25.391$; p -value < 0.01].

was 85% and negative predictive value was 96.6% (Table 2, figure 2).

This showed that posttest odds for HCC presence increased 4.1 fold when liver stiffness is larger than 25 kPa whereas the odds decrease to less than one-fifth when stiffness is smaller than 10 kPa. Multivariate analysis showed a positive direct correlation between the liverstiffness & the presence of HCC & the number of focal lesions ($P < 0.001$).

Univariate analyses showed that the risk of HCC increased in accordance with SSLR; older age, male gender, clinical cirrhosis, lower serum albumin level, higher total bilirubin level, higher ALT & AST levels & lower prothrombin $p < 0.001$. It also showed high significant negative correlation with PC% and albumin.

DISCUSSION

The population of Egypt has a heavy burden with HCV (14.7%), so it has been projected that the number of cases of hepatocellular carcinoma will continue to increase for the next three decades^[16]. In Egypt, HCC was reported to account for about 4.7% of chronic liver disease (CLD) patients^[17]. Precise staging of hepatic fibrosis is paramount as fibrosis is the most important predictor of disease outcome^[18].

Histological examination of a liver specimen obtained by percutaneous biopsy has traditionally been considered as the gold standard for evaluating hepatic fibrosis^[19]. It has up to 20% error rate and it can spread malignant cells in case of HCC as a sequel of HCV^[20].

Diagnosis of HCC is based mainly on discovering of a hepatic focal lesion on abdominal ultrasound, followed by confirmation by serum AFP > 200 ng/dL or higher, and presence of hepatic focal lesion on triphasic spiral CT with characteristic Enhancement^[15].

Transient elastography (TE) using Fibroscan is a novel non-invasive method for assessment of liver fibrosis, showing high concordance with values acquired by the golden standard which is liver biopsy^[21]. In addition, TE can be used in evaluation of cases with portal hypertension^[22], cases with recurrent hepatitis C after living donor liver transplantation^[23] and it can be used in HCC patients' assessment after radiofrequency ablation^[24]. We tried to prove if transient elastography could be used as a tool for identifying chronic HCV patients who are at higher risk of developing HCC.

As regards the epidemiological features of our patients, there was male sex predominance in HCC patients and this went hand in hand with the study that was done by Liovet *et al.*^[25] (2003) as well as by El Serag and Rudolph, (2007)^[26] who reported that the degree of liver fibrosis, age and male sex to be the risk factors for HCC development. In our study, there was significant difference in the albumin level and the platelet count between the two groups as $p < 0.05$ and this was different from the results obtained by Velazquez *et al.*^[27] (2003) who reported that albumin and platelet count were not retained as significant factors.

Serum AFP was measured in all patients, the sensitivity of AFP in detecting HCC at the level of 200 ng/dL was very low (22%), however the specificity was very high (100%). The positive predictive value was 100% while the negative predictive value was 56.18%. These results were very similar to the results concluded by Nguyen *et al.*^[28] (2002) in which the sensitivity was 32% and the specificity was 100%; so that EASL guidelines for HCC in 2012 removed AFP as a screening method for HCC^[29].

In the present work, liver stiffness measurements were taken in all 250 patients by transient elastography using Fibroscan. Liver stiffness was expressed as a continuous variable so that TE is a very promising tool for the early detection of cirrhosis which is an advantage over

the liver biopsy especially in the transitional zones such as F0:F1, F1:F2 and F3:F4 because of the non cirrhotic liver at F3 in particular, still possesses a definitive risk of HCC development^[30].

TE may be able to detect the risk of HCC development in cirrhotic patients, as there is a significant correlation between LSM and the fibrosis stage in CHC patients because stiffness of the tissues depends on the presence of fibrous tissue as well as the presence of the fibrous septa within the normal liver architecture^[31]. This correlation is not affected by the activity grade^[32]. Stiffness range was 2.90-39.80 kpa among HCV patients (GI) with a mean of 8.95±7.27 kpa while it was 8.4-75.00 kpa in HCC patients (GII) with a mean of 31.10±16.05 kpa. These results come very close to the results of the study carried by Masuzaki *et al*^[10] (2008) where the mean for HCV patients was 9.85 ±6.95 kpa, while in the HCC group it was 22.3±14 kpa.

Moreover, TE may be able to differentiate risk of HCC among cirrhotic livers, as there is a significant correlation between LSM and fibrosis stage in patients with chronic HCV^[31]. This observation is consistent because stiffness of tissues largely depends on their molecular building blocks (collagen) and on the microscopic structural organization of these blocks (septa) and this correlation is not affected by the activity grade^[32]. Stiffness range was 2.90-39.80 kpa among HCV patients (GI) with a mean of 8.95±7.27 kpa while it was 8.4-75.00 kpa in HCC patients (GII) with a mean of 31.10±16.05 kpa. These results come very close to the results of the study carried by Masuzaki *et al*^[10] (2008) where the mean for HCV patients was 9.85±6.95 kpa, while in the HCC group it was 22.3±14 kpa.

Using receiver operator characterizing (ROC) curve, LSM showed a high sensitivity (97%) and specificity (84%) for detecting HCC at the level of >12 kpa with a positive predictive value of 85% and a negative predictive value of 96.6%. The area under the curve (AUC) was 0.941 ($p<0.1$). Again these results come very close to the results obtained by Masuzaki *et al*^[10] where the AUC was 0.805.

In clinical practice, however LSM will not be used as a diagnostic test of HCC, but rather as an indicator of the risk of HCC. In this aspect, Stratum Specific Likelihood Ratio (SSLR) is better than a fixed cutoff value. For continuous scores such as liver stiffness, SSLRs retain as much information as possible by deriving multiple level indices.

In our study the posttest odds increased to 4.1 fold when the liver stiffness is >25 kpa while decreased to less than one fifth when the liver stiffness is <10 kpa. Which was similar to the study that was carried by Masuzaki *et al*^[10] who reported that the posttest odds increased to five folds when LSM was >25 kpa and decreased to one fifth when the LSM was <10 kpa.

Our results represented a positive significant correlation with the number of FHLs, to our knowledge, no published data discussed this issue specifically, but this can be attributed to the changes that occurred to the elasticity of the liver as a result of increased the amount of fibrous and malignant cells within the liver.

CONCLUSION

SSLR could be a useful predictor of HCC development in patients with CHC. Patients expressing fibroscan scoring >25 kPa and SSLR >4.1 are in need for meticulous follow up by imaging examinations.

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