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RESEARCH ARTICLE

Use of Enhanced liver fibrosis test (ELF) in Egyptian patients with chronic hepatitis C virus (HCV) infection to identify severity of liver fibrosis

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Abstract

Objective: To evaluate the role of enhanced liver fibrosis test (ELF) in identification of the stage of liver fibrosis in chronic HCV patients.

Patients and Methods: This study included 75 chronic HCV patients and 25 age and sex matched healthy subjects (control group). All patients were investigated by ELF, fibroscan and liver biopsy. ELF markers included hyaluronic acid (HA), tissue inhibitor of metalloproteinase (TIMP) and procollagen III amino terminal peptide (PIIINP).

Results: HA and TIMP in HCV patients showed no significant difference from controls, while PIIINP in HCV patients showed a highly significant difference from controls ($P>0.001$). The value of ELF test was elevated in HCV patients with a significant difference from controls ($P<0.05$). In HCV patients, there was a highly significant difference among the stages of fibrosis as regards PIIINP, TIMP and ELF test ($P<0.001$). There was a significant correlation between ELF score and AST, ALT and all ELF markers ($P<0.001$). The sensitivity of ELF test in relation to biopsy is 85.3%, while the sensitivity of fibroscan test in relation to biopsy is 54.7%.

Conclusion: ELF test could be a useful screening tool for detection of the stage of liver fibrosis in HCV patients. This test is more sensitive than fibroscan in assessment of the severity of liver fibrosis.

INTRODUCTION

It is estimated that 150-200 million people, or nearly 3% of the world population, are living with chronic hepatitis C. About 3-4 million people are infected per year, and more than 350,000 people die yearly from hepatitis C – related diseases ***(Alter, 2007 and Hanafiah et al. 2013).***

Egypt has the highest HCV prevalence in the world (14.7%). Therefore, HCV infection and its complications are among the leading public health challenges in Egypt today ***(Mahmoud et al. 2013 and Cuadros et al. 2014).***

Liver fibrosis results from chronic damage to the liver in conjunction with the accumulation of extra cellular matrix (ECM) proteins, which is a characteristic of most types of chronic liver diseases ***(Friedman , 2003)***

The main cause of liver fibrosis in developing countries is chronic HCV infection. The accumulation of ECM proteins distorts the hepatic architecture by forming a fibrous scar, and the subsequent development of nodules of regenerating hepatocytes defines cirrhosis. Cirrhosis produces hepatocellular dysfunction and increased intrahepatic resistance to blood flow, which result in hepatic insufficiency and portal hypertension, respectively **(*Gines et al., 2004 and Zhang et al. 2005).***

Liver biopsy is the gold standard test to assess the nature and severity of liver disease. Due to limitations of accuracy and patient hazard of liver biopsy, non-invasive methods have been sought to provide information on liver

fibrosis, including radiological investigations as Transient Elastography and laboratory investigations as the Enhanced liver fibrosis (ELF) test **(*Parkes et al.,2011*).**

This study was designed to evaluate the ELF performance in the identification of the stage of liver fibrosis which is usually asymptomatic, in order to intervene before significant damage to the liver in patients with chronic HCV infection.

Patients and Methods

This study included 75 patients with chronic HCV infection as evidenced by HCV Ab and HCV PCR. Their ages ranged from 19 to 68 years with a mean±SD of 39.43±12.6 years. Patients were collected from outpatient clinics and inpatient sections of Internal Medicine Department, Zagazig University Hospitals during the period from November 2013 to March 2015. The study also included 25 ages and sex matched healthy subjects (control group).

Informed written consents were obtained from all participants and the study was approved by the local Ethics Committee.

Patient inclusion criteria:

Patients with chronic HCV infection without cirrhosis as classified by laboratory and histological evidence were included.

Patient exclusion criteria:

1. Histological or laboratory diagnosis of cirrhosis as evidenced by any one of the following:
    - Platelets count less than the lower limit of normal (LLN).
    - Serum albumin less than LLN.
    - Ultrasound evidence of cirrhosis (coarse echo texture, irregular outline to liver, splenomegaly).
2. Any episode of hepatic decomposition compatible with cirrhosis including:
    - Encephalopathy, variceal bleeding and ascites.
    - Established diagnosis of hepatocellular cancer.

Patients were subjected to:

I-Laboratory investigations including:

- Complete blood picture (performed on Sysmex KX 21N).
- Prothrombin time, prothrombin concentration and International Normalization Ratio (INR) were performed on Sysmex CA 1500.
- Liver and kidney functions tests were performed on Cobas Integra 400 plus (Roche diagnostics).
- Viral markers (HCV Ab and HBsAg) were performed on Cobas e 411(Roche diagnostics).
- Polymerase chain reaction (PCR). HCV RNA extraction was performed on cobas ampliprep using its detecting reagents while amplification and detection on Real Time PCR(RT PCR) using Cobas Taqman 48(Roche diagnostics).
- Enhanced liver fibrosis test (ELF):
  3ml of venous blood was collected from each patient on plain vacuntainer tube then let stand for 30 minutes to clot. Sera were separated by centrifugation at 3000 rpm for 5 minutes then sera were separated and then frozen at (-80°c) till analysis .The test included:
  1- Hyaluronic acid (HA) (Normal range=10-100ng\ml)
  2-Procollagen III amino terminal peptide (PIIINP) (Normal range=2-4ng\ml)
  3-Tissue inhibitor of metalloproteinase (TIMP-1) (Normal range=80-500ng\ml).
  The test was performed using ADVIA Centaur XP Immunoassay Systems by direct chemiluminescence method. The results are computed using the following formula:

ELF score = 2.278 + 0.851 ln(CHA) + 0.751 ln(CP3NP) + 0.394 ln (CTIMP1).

II- Radiological investigation including:

- Abdominal ultrasound.
- Vibrating controlled Transient Elastography FibroScan® (EchoSense, Paris, France) was performed for HCV patients only.

III- Histopathological investigation including:

Liver biopsy was done for HCV patients only. Histopathological diagnosis classifies the severity of fibrosis into four stages, F1 to F4. F1 is mild fibrosis, F2 is moderate fibrosis, F3 is severe fibrosis and F4 is the final stage- cirrhosis **(*Batts and Ludwig 1995*)**.

The controls were subjected to:

Laboratory investigations including:

- Complete blood picture.
- Prothrombin time and INR.

- Liver and kidney functions tests.
- Viral markers (HCV Ab and HBs Ag)
- Enhanced liver fibrosis test (ELF).

Statistical Analysis: The data were tabulated and statistically analyzed using Microsoft Office Excel 2010, and Statistical Package for Social ***Sciences version 20*** (SPSS: An IBM Company. Student "t" test was used to compare between two groups regarding quantitative data. ANOVA test was used to analyze the differences between more than two independent group means. Pearson's correlation co-efficient was used to measure the degree of linear dependence between two variables. Sensitivity test (also called the *true positive rate*) measures the proportion of actual positives which are correctly identified. P value was assumed to be significant at <0.05 and highly significant at <0.001.

Results:

There was a significant elevation in liver enzymes (ALT and AST) in HCV patients when compared with the control group. Two ELF markers (HA & TIMP) in HCV patients showed no significant difference from the control group, while PIIINP in HCV patients showed a high significant difference from the control group (P<0.001). The value of ELF test was elevated in both groups with a significant difference from the control group (P<0.05). These findings are shown in Table (1).

ANOVA test of HCV patients showed non significant difference among the stages of fibrosis in HA results, while there was a highly significant difference among the stages of fibrosis in PIIINP, TIMP and ELF results (P<0.001) as shown in Table (2).

There was a significant correlation between ELF score and AST, ALT and all ELF markers (P<0.001) as shown in Table (3).

The sensitivity of the ELF test in relation to the biopsy (the gold standard test) is 85.3%, while the sensitivity of fibroscan test in relation to the biopsy is 54.7% (Table 4).

Table (1): Demographic and laboratory findings in the studied groups

	HCV patients (n = 75)	Controls (n = 25)	P-value	Sig.
Sex: Male: No (%) Female: No (%)	67 (89.3%) 8 (10.7%)	22(88%) 3(12%)	>0.05	NS
Age M±SD (Years)	39.43±12.6	38.9±11.8	>0.05	NS
ALT M±SD (IU/L)	47.6±20.9**	24.2±1.19	<0.001	HS
AST M±SD (IU/L)	48.2±21.7**	23.9±1.04	<0.001	NS
Total bilirubin M±SD (mg/dl)	0.63±0.32	0.56±0.13	>0.05	NS
Direct bilirubin M±SD (mg/dl)	0.13±0.04	0.12±0.03	>0.05	NS
Albumin M±SD (g/dl)	4.5±0.71	4.9±0.87	>0.05	NS
Creatinine M±SD (mg/dl)	0.9±0.24	0.9±0.27	>0.05	NS
Platelet M±SD	229±64	246±77	>0.05	NS
INR M±SD	1±0.04	1±0.08	>0.05	NS
HA M±SD (ng/ml)	46.8± 34.8	44.12±22.4	>0.05	NS
PIIINP M±SD (ng/ml)	11.1±7.2**	2.8±0.52	<0.001	HS
TIMP M±SD (ng/ml)	198±72	174±69	>0.05	NS
ELF M±SD	8.4±1.1	6.9±0.91	<0.05	S

NS: non significant S: Significant HS: highly significant

Table (2): ANOVA test of HCV patients

	No	Mean±SD	Minimum	Maximum	F	P
HA						
F_1	48	42.6±34.4	7	256	1.9	>0.05
F_2	23	45.1±32.7	11	198		
F_3	4	46.3±0.96	45	47		
PIIINP						
F_1	48	10.2±4.7	4	22	48	<0.001
F_2	23	14.2±3.5	6	21		
F_3	4	29.8±3.2	22	37		
TIMP						
F_1	48	189±47	104	325	32	<0.001
F_2	23	224±54	117	386		
F_3	4	368±18	341	394		
ELF						
F_1	48	8.1±1.2	7.1	12.4		
F_2	23	9.7±1.1	7.4	11.8	21	<0.001
F_3	4	13.1±0.8	12.4	13.4		

Table (3): Correlation between ELF and other laboratory investigations

	ELF Pearson correlation	Sig(2-tailed)
ALT	0.486	<0.001
AST	0.502	<0.001
Total bilirubin	0.132	>0.05
Direct bilirubin	0.147	>0.05
Albumin	-0.136	>0.05
Creatinine	0.168	>0.05
Platelet	-0.174	>0.05
INR	0.204	>0.05
HA	0.472	<0.001
PIIINP	0.595	<0.001
TIMP	0.573	<0.001

Table (4): Validity tests between biopsy versus ELF and fibroscan

	Biopsy (+ve)
ELF	
<7.7: No (%)	11(14.7%)
>7.7: No (%)	64(85.3%)
Total	75(100%)
Fibroscan	
-ve : No(%)	34(55.3 %)
+ve : No(%)	41(54.7%)
Total	75(100%)

Sensitivity of ELF test=85.3% and Sensitivity of fibroscan=54.7%

Discussion

Complete evaluation of a patient with diffuse liver diseases requires clinical evaluation, biological evaluation and histopathological examination-liver biopsy, for the grading and staging of the liver disease. The clinical evaluation is often irrelevant, only the presence of spider naevi on the anterior thorax or an enlarged and firmer liver could suggest that the patient already has liver cirrhosis (suspicion that has to be confirmed by further tests). The biological evaluation by means of the usual tests is also often irrelevant, especially in chronic hepatitis C (CHC), known to induce sometimes severe hepatic lesions, while the aminotransferases are normal or only slightly elevated. Thus, it is considered that the liver biopsy has a key role for the diagnosis and follow-up of chronic diffuse liver diseases especially for the staging of chronic hepatitis C ***(Saadeh et al., 2001*)*.**

However, many studies clearly highlight several drawbacks of liver biopsy, including variable accessibility, high cost, sampling errors and inaccuracy due to inter- and intra-observer variability of pathologic interpretations. ***(Sebastiani and Alberti, 2006).***

In recent years, interest in identifying and describing liver fibrosis by using non invasive markers has been on the rise. Serum markers of liver fibrosis offer cost effective alternative to liver biopsy for both patients and clinicians. In addition to being less invasive, there are practically no complications, little or no sampling errors and small observer related variability. Moreover, measurements may be performed repeatedly, thus, allowing for a dynamic monitoring of fibrosis ***(Zhou and Lu, 2009).***

Enhanced liver function test (ELF) is a non- invasive diagnostic test that uses a combination of direct serum markers to identify the stage of liver fibrosis even in patients without symptoms. Direct serum markers are proteins that reflect extracellular matrix metabolism/ degradation produced as a result of the fibrogenic process ***(Badra et al., 2010).***

This study was carried out to evaluate the ELF performance in the identification of the stage of liver fibrosis which is usually asymptomatic aiming to intervene before significant damage to the liver in patients with chronic HCV infection.

In the present study, it was found that two ELF markers (HA &, TIMP) in HCV patients showed no significant difference from the control group, while PIIINP showed a highly significant difference from the control group ($P<0.001$). These results were discordant with ***Martinez et al., (2011)*** who compared the diagnostic performance of different non invasive tests including ELF score in prediction of the liver fibrosis stage in HCV patients. In their study, all the ELF markers were significantly elevated. This may be attributable to that 67% of their patients showed significant fibrosis ($F\geq2$) while 36% of our patients (27 out of 75 patients) showed significant fibrosis. Also our results were discordant with ***Yasser et al., (2013)*** where both HA and PIIINP were significantly elevated and TIMP was highly significantly elevated. This also may be attributable to that 70% of their patients showed significant fibrosis.

The results of our study showed that in HCV patients, the mean value of ELF test was elevated (8.4). This was in agreement with ***Martinez et al., (2011)*** who found that the mean value of ELF test was 8.2 in their study.

ANOVA test for HCV patients showed no significant difference among stages of fibrosis in hyaluronic acid (HA) results, while there was a high significant difference among stages of fibrosis in PIIINP, TIMP and ELF results ($P<0.001$). These results were concordant with ***Martinez et al., (2011*)** who concluded that ELF can accurately identify significant fibrosis and cirrhosis in HCV patients. Also these results were in agreement with ***Kristin et al.,(2012)*** who showed that the ELF score reveals similar diagnostic accuracy to predict relevant ($\geq F2$) or advanced stages of fibrosis and appear to be less discriminative in lower degree of fibrosis. In their study, they evaluated ELF score in comparison to transient elastography in prediction of different stages of liver fibrosis in patients with chronic liver disease.

In our study, Pearson correlation showed a significant correlation between ELF score and level of AST and ALT. These results were concordant with ***Kristin et al., (2012)***. However these results were discordant with ***Yasser et al., (2013)*** who revealed that there were no significant correlation between level of ALT and AST and the stage of fibrosis. They stated that the increase in ALT levels is related to mitochondrial dysfunction and reduced clearance of ALT by hepatic sinusoidal cells rather than the degree of fibrosis.

Validity tests between biopsy versus ELF and fibroscan showed that the sensitivity of ELF for fibrosis detection was 85.3% while that of fibroscan was 54.7% in the current study. This was in agreement with ***Kristin et al. (2012)*** who revealed that sensitivity of ELF was higher than that of fibroscan in detection of fibrosis.

In conclusion, our results showed that ELF test could be a useful screening tool for detection of the stage of liver fibrosis in HCV patients. This test is more sensitive than fibroscan in assessment of the severity of liver fibrosis.

Further larger studies are recommended to accurately determine whether to use ELF biomarkers instead of liver biopsies. Further researches are also required to asses ELF score in many liver diseases rather than HCV e.g. fatty liver, autoimmune hepatitis and HBV.

References

Alter MJ (2007): Epidemiology of hepatitis C virus infection. World J Gastroenterol, 13 (17): 2436–2441.

Badra G, Lotfy M, El-Refaie A, et al. (2010): Significance of serum matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 in chronic hepatitis C patients. Acta Microbiol Immunol Hung, (1):29-42.

Batts KP and Ludwig J (1995): Chronic hepatitis: An update on terminology and reporting. Am J Surg Pathol 19:1409-1417.

Cuadros DF, Branscum AJ, Miler FD, and Abu-Raddad LJ (2014): Spatial epidemiology of hepatitis C virus infection in Egypt. Hepatology 60(4):1150-1159.

Friedman SL (2003). Liver fibrosis - from bench to bedside. J. Hepatol. 38(Suppl. 1):S38–S53.

Gines, P, Cardenas A, Arroyo V and Rodes J (2004): Management of cirrhosis and ascites. N. Engl. J. Med. *(*350):1646–165.

Hanafiah MK, Groeger K, Flaxman AD and Wierma ST (2013): Global epidemiology of hepatitis C virus infection: New estimates of age-specific to HCV seroprevalence.
Hepatology 57(4):1333-1342.

Kristin W , Rosenberg W, Vaske B, et al., (2012): Biopsy-Controlled Liver Fibrosis Staging Using the Enhanced Liver Fibrosis (ELF) Score Compared to Transient Elastography. PLOS ONE \ www.plosone.org Volume 7 \ Issue 12 \ e51906.

Mahmoud YA, Mumtaz GR, Riome S et al. (2013): The epidemiology of hepatitis C virus in Egypt: A systematic review and data analysis. BMC Infectious Diseases 13:288.

Martinez, G. Fernandez VP, Gonzalez E et al. (2011): Assessment of liver fibrosis before and after antiviral therapy by different serum marker panels in patients with chronic hepatitis C. Alimentary Pharmacology and Therapeutics 33: 138-148.

Parkes J, Guha IN, **Roderick and Rosenberg (2011):** Enhanced Liver Fibrosis (ELF) test accurately identifies liver fibrosis in patients with chronic hepatitis C. Journal of Viral Hepatitis**.**Volume 18, Issue 1, pages 23–31.

Saadeh S, Cammell G, Carey WD et al. (2001): The role of liver biopsy in chronic hepatitis C.
Hepatology; (33): 196-200.

Sebastiani G and Alberti D (2006): Non invasive fibrosis biomarkers reduce but not substitute the need for liver biopsy. World J Gastroenterol,12(23):3682-3694.

Strader DB, Wright T, Thomas DL, Seeff LB (2004): Diagnosis, management, and treatment of hepatitis C. Hepatology; (39) 1147-1171.

Yasser N, Abu Shady M, Galal EM and Abd El Hamed M (2013): Performance of diagnostic biomarkers in predicting liver fibrosis among hepatitis C virus-infected Egyptian children. Rio de Janeiro, Vol. *108*(7): 887-893

Zhang M, Sun XD, and Mark SD. (2005): Hepatitis C virus infection, Linxian, China. Emerg Infect Dis, (11): 17–21.

Zhou K, and Lu LG (2009): Assessment of fibrosis in chronic liver diseases. Journal of Digestive Diseases, 10(1):7-14.