Liver stiffness measurement using transient elastography is affected by serum total bilirubin in medicalcholestasis

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Abstract: Background: Transient elastography (TE) is a non-invasive and reproducible tool to assess liver fibrosis and cirrhosis. However, it remains to be determined if cholestasis interferes with fibrosis assessment. **Aim:** To determine the effect of increased serum total bilirubin on liver stiffness measurement in patients with medicalcholestasis. **Methods:** Thirty consecutive patients with cholestasis (total serum bilirubin > 10 mg/dl) were prospectively included. Blood samples were collected for assessment of liver functions tests and TE was done initially and after resolution of cholestasis (decrease in serum bilirubin level <3mg/dl). Patients with high BMI which could affect fibroscan were excluded. For determination of the etiology of cholestasis, a detailed physical examination and history taking were performed in all patients. Exclusion of obstructive jaundice was established by abdominal ultrasonography or magnetic resonance cholangiopancreatography (MRCP). **Results:** Patients were 43.83 ± 7.64years old and males were 22 patients (73.3%). Patients with F2 represented 6.7% (2 patients), patients with F3 represented 20% (6 patients) while LSM of 22/30 of patients reached cirrhotic level (F4).There was statistically significant change in LSM value after decrease in bilirubin level ($P< 0.01$). The mean value of LSM at the time of inclusion in the study was 29.01±18.84kPa and the mean value of LSM after resolution of cholestasis was 17.57±11.15 kPa. The decrease in the mean level of LSM value was statistically significant ($P< 0.01$). The mean value ± SD of total bilirubin level at baseline was 15.66 ± 6.19 mg/dl and the mean value ± SD of total bilirubin level after the decrease in its level was 2.33 ± 0.61 mg/dl. The decrease in the mean level of total bilirubin level was statistically significant ($P< 0.01$). **Conclusion:** TE has not demonstrated reliable diagnostic accuracy in patients with cholestasis.

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1. Introduction

Transient elastography (TE) is a noninvasive method for assessing the degree of liver fibrosis. It is rapid (less than 5 min), painless, noninvasive and reproducible. The risk of sampling error is significantly lower as it acquires information from a much larger portion of the tissue compared with liver biopsy. However, increased liver stiffness is not always a surrogate of fibrosis (the presence of significant necroinflammation or extrahepatic cholestasis may increase liver stiffness values in the absence of fibrosis) ***(1).*** Liver stiffness measurement can be difficult in obese patients or in those with narrow intercostal space and impossible in patients with ascites***(2)***. Several studies showed that the technique is reproducible, has low interobserver variation and correlates well with the severity of fibrosis in patients with chronic hepatitis C (CHC) and HCV-related cirrhosis ***(3).*** In all the studies, the degree of liver fibrosis assessed by fibroscan was compared to the histopathological fibrosis stage assessed by Metavir group scoring system ***(4)*** (F0, no fibrosis; F1, portal fibrosis without septa; F2, few septa; F3, numerous septa without cirrhosis; or F4, cirrhosis). ***Ziol et al. (5)*** and ***Castera et al. (6)*** demonstrated that fibrosis was the only parameter significantly correlated to liver stiffness (LS). However, it remains to be determined if other liver such as extrahepatic cholestasis interfere with fibrosis assessment because liver stiffness is indirectly measured by the propagation velocity of an ultrasound wave within the liver***(7).*** Extrahepatic cholestasis is caused by benign diseases or carcinomas of the biliary tract or pancreas. The increase of LS in extrahepatic cholestasis may differ between benign diseases and carcinomas, while the differences of increase of LS among the causes of obstruction have not been studied ***(8).*** Also, the increase of LS in medical cholestasis has not been evaluated. **Aim of the work:** To determine the effect of increased serum total bilirubin on liver

stiffness measurement in patients with medical cholestasis.

2. Patients and Methods

Thirty consecutive patients with medical cholestasis (with total serum bilirubin > 10 mg/dl) who were referred to the clinical hepatology department, National Liver Institute, Menoufiya University, Egypt and theywere prospectively included in the study. For determination of the etiology of cholestasis, a detailed physical examination and history taking icluding past history of exposure to hepatotoxic drugs were performed in all patients. Exclusion of obstructive jaundice was established by abdominal ultrasonography or magnetic resonance cholangiopancreatography (MRCP). Blood samples were collected and TE was done initially (LSM1) and after resolution of cholestasis (LSM2) [decrease in serum bilirubin level <3mg/dl].Initial laboratory tests included measurements of total serum bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), and alkaline phosphatase (AP). These tests were done using Cobas Integra 400, Hoffman La Roche Company, Switzerland. Prothrombin time and concentration were assessed using Thromborel S, Behring fibrin timer II, Behring Inc., Germany. ***Complete blood counts*** were measured using **Sysmex instrument KX-21, Sysmex Inc., Japan.** ***Viral markers*** (HCV Ab, HBs Agand HAV IgM) were detected by means of **a third generation ELISA.** Quantitative real time PCR was done for patients with acute HCV, HBV infections. The diagnosis of acute HCV infection generally requires testing of serum for both antibody to HCV (anti-HCV) and for HCV RNA level. A sensitive quantitative HCV RNA assay is recommended for diagnosis. The differentiation of acute from chronic HCV infection depends on the clinical presentation: namely the presence of symptoms or jaundice and whether or not there was a prior history of ALT elevation and its duration (9). Acute hepatitis B was defined by positive immunoglobulin (Ig) M antibody to hepatitis B virus core antigen (anti-HBc) and negative anti-HBs. The HBV DNA level was also measured to support the diagnosis of acute hepatitis B (10). Diagnosis of acute HAV infection is based on the detection of anti-HAV IgM antibodies or HAV RNA. The presence of HAV IgG antibodies can indicate previous HAV infection (11). Sepsis is the syndrome of the systemic inflammatory response to infection and it is defined by the presence of at least two of the following criteria: (1) altered temperature, (2) elevated respiratory rate or hyperventilation, (3) tachycardia, and (4) altered white blood cell count (high, low, or immature forms) (12).

Serum copper, serum ceruloplasmin and 24 h urinary copper were done for diagnosis of Wilson disease. Patients with high BMI >35 kg/m^2 were excluded.

Statistical analysis

A statistical analysis was performed using SPSS, version 22 (SPSS Inc., Chicago, IL, USA); results were expressed as mean ± standard deviation (SD). Paired t test was done to detect mean and standard deviation of normally distributed pre and post values of the same variable of the same group of patients and *p*-value < 0.05 was considered significant. Wilcoxon test was done to detect mean and standard deviation of not normally distributed pre and post values of the same variable of the same group of patients and *p*-value < 0.05 was considered significant. Spearman's correlation test was done to study correlation between one qualitative variable and one quantitative variable or two quantitative variables of not normally distributed data and *p*- value less than 0.05 was considered significant.

3. Results

Patients were 43.83 ± 7.64years old in the range of 29-57 years and males were 22 patients (73.3%). Patients with F2 represented 6.7% (2 patients), patients with F3 represented 20% (6 patients) while LSM of 22/30 of patients reached cirrhotic level (F4). Descriptive data and patient's characteristics were shown in table 1.

The decrease in the mean level of LSM value was statistically significant after the decrease in bilirubin level (t= 3.82; *P*< 0.01). Patients with F2 represented 6.7% (2 patients), patients with F3 represented 20% (6 patients) while patients with F4 represented 73.3% (22 patients). LSM of 28/30 of patients showed significant fibrosis while 22/30 of patients reached cirrhotic level. Patients with F1 represented 6.7% (2 patients), patients with F2 represented 20% (6 patients), patients with F3 represented 33.3% (10 patients) while patients with F4 represented 40% (12 patients). LSM of 22/30 of patients showed significant fibrosis while 12/30 of patients reached cirrhotic level.Spearman correlation between LSM and laboratory variables in all studied patientswas done as shown in table 2.

Table (1):- Descriptive data and patient's characteristics.

Studied variables	Mean ± SD (n=30)	Range
Age (in years)	43.83 ± 7.64	29 – 57
Body mass index (kg/m^2)	27.75 ± 1.95	24.81 – 32.48
LSM1 (kPa)	29.01 ± 18.84	6.3 – 75
LSM2 (kPa)	17.57±11.15	4.2 –69
ALT (U/L)	175.9 ± 52.6	98 – 292
AST(U/L)	118.1 ± 62.5	40 – 245
Albumin (gm/dl)	3.63 ± 0.43	2.9 – 4.7
Initial total bilirubin (mg/dl)	15.66 ± 6.19	10-50
Total bilirubin after resolution of cholestasis (mg/dl)	2.33 ± 0.61	1 – 3
Gender: **Male** **Female**	 22 (73.3%) 8 (26.7%)	
Etiological classification **Drug induced cholestasis** **Sepsis** **Acute hepatitis C** **Acute hepatitis B** **HCV&HBV coinfection** **Wilson disease**	 11 (36.7%) 4 (13.3%) 6(30 %) 3 (10 %) 2 (6.7%) 4 (13.3%)	

Table (2):- Spearman correlation between LSM and laboratory variables in allstudied patients.

		LSM1	LSM2
Age (in years)	R	0.055	0.302
	p- value	> 0.05	> 0.05
BMI kg/m^2	R	-0.018	
	p- value	> 0.05	
ALT U/L	R	-0.618	-0.699
	p- value	< 0.01*	< 0.01*
AST U/L	R	0.070	-0.023
	p- value	> 0.05	> 0.05
GGT U/L	R	0.071	-0.021
	p- value	> 0.05	> 0.05
ALP U/L	R	-0.133	-0.345
	p- value	> 0.05	> 0.05
Albumin gm/dl	R	-0.514	-0.672
	p- value	< 0.01*	< 0.01*
Initial total bilirubin mg/dl	R	0.383	
	p- value	< 0.05*	
Total bilirubin after resolution of cholestasis mg/dl	R		0.572
	p- value		< 0.01*
PC %	R	-0.524	-0.616
	p- value	< 0.01*	< 0.01*
INR	R	0.547	0.608
	p- value	< 0.01*	< 0.01*

ALT: Alanine Aminotransferase; **AST:** Aspartate Aminotransferase; **GGT:** Gamma-Glutamyl Transpeptidase; **ALP:** Alkaline Phosphatase; **INR:** International Normalized Ratio; **PC:** Prothrombinconcentration; **BMI:** Body Mass Index

4. Discussion:

To our knowledge, no published data exist on the assessment of TE in patients with medical cholestasis (with no mechanical obstruction). In the present study, Males represented 73.3% (22 patients). The underlying disease was established according to standard criteria using laboratory tests, ultrasound, CT imaging and ERCP. Initial serum bilirubin levels ranged from 10 to 50 *mg/dl* (mean 16.36 ± 8.09 *mg/dl*). The mean value ± SD of LSM 1 by fibroscan was 29.01 ± 18.84 *kPa* ranging from 6.3 to 75 *kPa*, and the mean value ± SD of LSM 2 after resolution of cholestasis was 17.57 ± 11.15 *kPa* ranging from 4.2 to 69 *kPa*. The drop in the mean level of LSM values was statistically significant ($P< 0.01$). Nevertheless, LS fell significantly from 75 to 19.5 *kPa* but remained above the cut-off value for liver cirrhosis in 12 patients. Initially elevated LS values in all patients decreased in 25 out of 30 patients. Two of them presented with sepsis. One patient presented with HBV & HCV co-infection. The last 2 patients presented with HCV reactivation. Rapid progression to cirrhosis may play a role. Progression of LS is predictive of poor outcome. The mean value ± SD of initial total bilirubin level in all patients was 15.66 ± 6.19 mg/dl and the mean value ± SD of total bilirubin level after the decrease in its level was 2.33 ± 0.61 mg/dl. The decrease in the mean level of total bilirubin level was statistically significant ($P< 0.01$). Our study revealed that there was significant positive correlation between total bilirubin level 1 and LSM1 value ($r = 0.383$; $P< 0.05$). Also, there was positive correlation between LSM2 and total bilirubin after resolution of cholestasis ($r = 0.572$; $P < 0.01$). However, serum ALT level was negatively correlated neither with LSM 1 or LSM 2. **Corpechot *et al.* (13)** evaluated TE in 101 patients with chronic cholestatic diseases, liver stiffness measurements very accurately identified patients with advanced fibrosis and cirrhosis. This is relevant because the prognosis of chronic cholestatic diseases depends, at least in part, on the extent of fibrosis inthe liver.

Millonig et al. (7) found that bile duct ligation for 120 minutes on pigs led to a significant swelling of the liver and a tightly palpable gall bladder as compared to humans. LS values doubled during bile duct ligation and reached values suggesting F3 fibrosis. After removal of the bile duct ligation and a recovery period of 30 minutes, LS values returned to almost normal values around 6.1 *kPa*. ***Harata et al. (8)*** demonstrated that the elevation of LS significantly correlated positively with total bilirubin levels and negatively with ALT levels. These correlations were also noted when the patients with carcinomas and those with benign diseases were separately analyzed. Decrease of LS after biliary drainage significantly correlated with decrease of total bilirubin levels. Also, **Trifan *et al.* (14)** reported that extrahepatic cholestasis that is caused by choledocholithiasis increases LS values, which correlate with serum total bilirubin levels ($r=0.691$; $P< 0.0001$). Significant reductions in LS and bilirubin levels are obtained after successful endoscopic sphincterotomy and stone removal.In our study, LSM was negatively correlated with ALT and AST ($P> 0.05$). The negative correlation between ALT levels and LS probably indicates the negative correlation between ALT levels and the hepatic hydrostatic pressure. The leakage of bile fluid from the bile canalicular lumen into the lateral intracellular space and the perisinusoidal space causes the damage of hepatocytes and may also reduce the hydrostatic pressure in the bile duct **(15).** The present study showed that cholestasis interferes with the determination of liver fibrosis by LSM and may erroneously suggest the presence of liver cirrhosis. The reasons underlying the high stiffness in studied patients with cholestasis are unknown but could be related to tissue swelling, inflammation, edema and liquid accumulation due to increased intracellular pressure due to impaired bile flow. The decrease in LSM value in patients with medical cholestasis needs further evaluation.

Recommendations :

TE has not demonstrated reliable diagnostic accuracy in medical cholestasis.

References :

1. Stella M, Gonzalo Crespo, Miquel Navasa, et al. (2011): Noninvasive assessment of liver fibrosis. *Hepatology; 53: 325-335.*
2. Sagir A, Erhardt A, Schmitt M, *et al.* (2008): Transient elastography is unreliable for detection of cirrhosis in patients with acute liver damage. *Hepatology; 47:* 592-595.
3. De Franchis R and Dell Era A (2007): Non-invasive diagnosis of cirrhosis and the natural history of its complications. *Best practice and Research clinical gastroenterology; 21, No 1: 3-18.*
4. Bedossa P and Poynard T (1996): An algorithm for the grading of activity in chronic hepatitis C. *Hepatology; 24: 289-293.*
5. Ziol M, Handra-Luca A, Kettaneh A, *et al.* (2005): Non-invasive assessment of liver fibrosis by measurement of stiffness in patients with chronic hepatitis C. *Hepatology; 41: 48-54.*
6. Castera L, Vergniol J, Foucher J, *et al.* (2005): Prospective comparison of transient elastography, Fibrotest, APRI and liver biopsy

for the assessment of fibrosis in chronic hepatitis C. *Gastroenterology; 128: 343-350.*
7. Millonig G, Reimann FM, Friedrich S, *et al.* (2008): Extrahepatic cholestasis increases liver stiffness (FibroScan) irrespective of fibrosis. *Hepatology; 48: 1718-1723.*
8. Harata M, Hashimoto S, Kawabe N, *et al.* (2011): Liver stiffness in extrahepatic cholestasis correlates positively with bilirubin and negatively with ALT. *Hepatology Research; 41: 423-429.*
9. Ghany MG, Strader DB, Thomas DL, *et al.* (2009): Diagnosis, Management, and Treatment of Hepatitis C: An Update. Hepatology, April; 49: 1335-1374.
10. Quint WG, de Bruijn I, Kruining H, *et al.* (1990): HBV-DNA detection by gene amplification in acute hepatitis B. Hepatology; 12: 653-656.
11. Tong MJ, el-Farra NS and Grew MI. (1995): Clinical manifestations of hepatitis A: recent experience in acommunity teaching hospital. J. Infect. Dis.; 171: Suppl 1: S 15-18.
12. Wong F, Bernardi M, Balk R, Christman B. (2005): Sepsis in cirrhosis: report on the 7th meeting of the International Ascites Club. Gut; 54: 718-725.
13. Corpechot C, El Naggar A and Poupon R (2006): Gender and liver: is the liver stiffness weaker in weaker sex? *Hepatology; 44: 513-514.*
14. Trifan A, Sfarti C, Cojocariu C, *et al.* (2011): Increasedliver stiffness in extrahepatic cholestasis caused by choledocholithiasis.*Hepat. Mon.; 11:372-375.*
15. Wiener SM, Hoyt RF Jr, Deleonardis JR, *et al.* (2000): Manometric changes during retrograde biliary infusion in mice.*Am J Physiol Gastrointest Liver Physiol; 279: G49-G66.*

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