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The Values of Hyaluronic Acid and as a Marker of Cirrhosis in Children with Chronic Liver Diseases

Nadia N Hassan[1] *BSc*, **Firyal H Al-Obaidi**[1] *BSc MSc*, **Hala S Arif**[2] *MBChB CABP*

[1]National center for drug control and research, [2]Dept. of Pediatrics, College of Medicine, Al-Nahrain University

Abstract

Background Hyaluronic acid (HA) is removed by the liver via sinusoidal cell adhesion molecules. This is impeded in fibrosis, leading to a rise in serum HA. As a noninvasive marker of fibrosis, HA may obviate the need for liver biopsy.

Objective To evaluate HA as a marker of hepatic fibrosis in unselected children undergoing liver biopsy or ultrasound.

Methods Fifty children aged 2-156 months diagnosed to have different types of chronic liver diseases (CLDs) and thirty healthy children aged 2-156 months were studied as controls were evaluated at the Teaching Hospital and Gastroenterology and Hepatology Center, Medical City, Baghdad, Iraq. The degree of severity of liver infection was assessed by liver biopsy or ultrasound. HA levels were measured using an ELISA.

Results The mean of HA level was 0.61± 0.32 ng/ml in the control group, 3.05± 1.11 ng/ml in patients with significant fibrosis and 1.18±0.86 ng/ml in patients with chronic liver diseases without significant fibrosis. Significant fibrosis was found in 31 out of 50 children with chronic liver disease, 20 of them were classified (METAVIR score) as cirrhotic liver. Seven out of 18 biopsies value of stage 4. Thirteen out of 32 ultrasounds described as having a coarsely textured liver. The sensitivity and specificity of estimated HA values in the diagnosis of liver cirrhosis were 87.1% and 94.74%, respectively.

Conclusion HA is a valid noninvasive predictor of histological fibrosis in children with CLD. It complements the thorough investigations of a child with CLD; however, it cannot at present replace histological examination to identify liver fibrosis. Further evaluation of HA is needed to ascertain the use of serial measurements in the targeted patient groups.

Key word Hyaluronic acid, chronic liver disease, liver fibrosis.

Introduction

A wide spectrum of chronic liver diseases can cause liver fibrosis (LF). Liver biopsy shows these histopathologic changes, determines the degree of fibrosis and is a guide to treatment decisions.

Many children each year require a liver biopsy under anesthesia. Liver biopsies, although generally a safe procedure, can be associated with serious morbidity and mortality, both from the procedure itself and the need for anesthesia in children [1,2]. Serious complications include hemoperitoneum, pneumothorax and bile leak [3] are seen in approximately 1% of liver biopsies [4]. Another important limitation of liver biopsy is inter- and intra observer variation among pathologists [5-8] and therefore, liver biopsies are only carried out if deemed essential.

Histological comparison of liver biopsies is limited by the lack of an ideal hepatic fibrosis

scoring system covering all spectrums of liver disease. The Ishak scoring system has been developed from the Histological Activity Index of Knowdell with modifications allowing for increased knowledge of etiology and histology [9]. Furthermore, the semi quantitative grading systems developed for histopathologic analysis do not reflect linearity of fibrosis deposition or actual matrix content [9,10].

Ultrasonography can provide a non-invasive prediction of liver histology which in moderate and severe steatosis and advanced fibrosis can be both highly sensitive and specific [11]. Ultrasonography is the most common modality used in the diagnosis and staging of hepatic fibrosis [12].

In recent years, interest in identifying and describing LF by using noninvasive surrogate markers has been on the rise. Serum markers of LF offer an attractive, cost effective alternative to liver biopsy for both patients and clinicians. In addition to being substantially 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 [13]. Hyaluronic acid (HA) is a high molecular weight glycosaminoglycan synthesized by mesenchymal cells, circulated by the lymphatic system and widely distributed in connective tissue [14]. It has a half-life of 5 to 6 minutes in plasma and a vital component in producing viscoelasticity in the extracellular matrix; it also lubricates the interstitial tissue. A small percentage of HA is locally metabolized while the greater part of it enters the blood through the lymphatic system and from there goes into the liver where it is immediately metabolized by the hepato-endothelial cells [15, 16].

The aim of the study is to determine the validity of serum HA as a marker of hepatic fibrosis in children with different types of chronic liver disease (CLD), undergoing a liver biopsy or ultrasound assessment.

Methods

This is a case-control study of fifty children with different types of CLDs**,** 18 of them have undergone liver biopsy at Gastroenterology and Hepatology Center, Medical city, Baghdad, Iraq during the period from Sep. 2011 to Feb. 2012. The METAVIR scoring system was applied to stage of LF. The other 32 were subjected to ultrasound evaluation at the same hospital to assess the liver texture as an indicator of significant LF [17,18]. Children with viral hepatitis were excluded from the study. No children were known at the time of sampling to have active juvenile idiopathic arthritis or any joint inflammation.

Conventional laboratory tests of liver function such as alanin aminotransferase (ALT), aspartate aminotransferase (AST), and total serum bilirubin (TSB) were undertaken for patients and controls within 48 hours of taking the samples. Blood samples for hyaluronic acid (HA) were separated and frozen at -20 °C before analysis. HA levels were measured using an enzyme-linked binding protein assay (Kit CUSABIO 2012, China).

Liver biopsies of 18 patients with different types of CLD were done by ultrasound guided technique with spring loaded needle, and then processed in the laboratories of Gastro-enterology and Hepatology Center. An experienced pathologist examined all the liver biopsy specimens during a single sitting. The pathologist was blinded to the results of the HA level and the underlying clinical diagnosis. METAVIR scoring system was applied. No or non significant fibrosis (0-1), significant fibrosis with (2-4). As for the ultrasound evaluated group, a coarse liver texture, especially when associated with irregularity of the liver surface, was regarded as indicator of significant fibrosis [14]. The subjects in this study were divided into three groups:

Group I: Children with different types of chronic liver disease with significant fibrosis.

Group II: Children with unselected chronic liver disease without significant fibrosis.

Group III: Healthy children (control).

All the data were analyzed using the Statistical package for social sciences (SPSS) software (v.17, SPSS, Inc., Chicago, USA). The student t-test was used to compare HA levels and the other biomarkers, in the groups of patients with and without significant fibrosis. Logistic regression analysis was undertaken with the presence and absence of significant fibrosis as the dependent variable and HA levels and standard liver functions tests (serum bilirubin, alanine transferase, and aspartate transaminase) as independent variables. To assess clinical applicability (sensitivity, specify and predictive values) a receiver operator curve was constructed and the area under the curve was calculated.

Results

Significant fibrosis was found in 31 of 50 children with CLDs. Twenty (40%) of them were classified (METAVER score) as cirrhotic liver, eleven (22%) have no cirrhosis and the rest 19 (38%) show no fibrosis (Figure 1). Seven out of 18 biopsies value of stage 4 and 13 of 32 ultrasound assessed group described as having a coarsely textured liver. The mean HA level was 0.61± 0.32 ng/ml in the control group, 3.05± 1.11 ng/ml in patients with significant fibrosis and 1.18 ± 0.86 ng/ml in patients with CLDs without significant fibrosis.

The results of study showed that HA, AST, ALT, and TSB levels were significantly increased in children with fibrosis compared to the control group ($p < 0.0001$; $p < 0.0001$, $p < 0.0001$; $p = 0.0001$, respectively). Similarly, AST/ALT ratio was significantly elevated in children with fibrosis compared to control group ($p = 0.0113$) as shown in table 1.

Table 1. Comparison between control group and patients with significant fibrosis

Parameters	Control Group N = 30 Mean ± SD	Patients with fibrosis N = 31 Mean ± SD	P value
Age (months)	68.8 ± 39.67	80.32±58.85	0.3726
Hyaluronic acid (ng/mL)	0.61 ± 0.32	3.05 ± 1.11	<0.0001
AST (IU/L)	6.47 ± 2.18	53.13 ± 43.96	<0.0001
ALT (IU/L)	7.53 ± 2.49	42.23 ± 32.53	<0.0001
AST/ALT ratio	0.93 ± 0.51	1.41 ± 0.86	0.0113
TSB (mg/dL)	0.62 ± 0.22	9.31 ± 10.79	0.0001

AST: Aspartate aminotransferase, ALT: Alanine transaminase, TSB: total serum bilirubin

As shown in the table 2, mean HA, AST, and ALT levels were higher in children without fibrosis compared to the control group ($p = 0.0108$; $p = 0.0001$; $p = 0.0007$, respectively) whereas, no significant differences in the AST/ALT ratio between the two groups ($p = 0.0834$).

Table (2): Comparison between control group and patients without fibrosis

Parameters	Control Group N = 30 Mean ± SD	Patients without fibrosis N = 19 Mean ± SD	P value
Age (months)	68.8 ± 39.67	60.37 ± 52.19	0.5521
Hyaluronic acid (ng/mL)	0.61 ± 0.32	1.18 ± 0.86	0.0108
AST (IU/L)	6.47 ± 2.18	48.21 ± 37.78	0.0001
ALT (IU/L)	7.53 ± 2.49	40.21 ± 34.76	0.0007
AST/ALT ratio	0.93 ± 0.51	1.45 ± 1.18	0.0834
TSB (mg/dL)	0.62 ± 0.22	7.39 ± 9.84	0.0077

AST: Aspartate aminotransferase, ALT: Alanine transaminase, TSB: total serum bilirubin

Table 3 illustrates comparison between CLDs children with and without fibrosis. The mean HA level was significantly higher in those with fibrosis as compared to those without (*P* < 0.0001) whereas, no significant differences was noticed in AST, ALT, AST/ALT and TSB levels between the two groups.

Table 3. Comparison between patients with and without fibrosis

Parameters	Patients with Fibrosis N = 31 Mean ± SD	Patients without fibrosis N = 19 Mean ± SD	P value
Age (months)	60.37 ± 52.19	80.32±58.85	0.2185
Hyaluronic acid (ng/mL)	1.18 ± 0.86	3.05 ± 1.11	<0.0001
AST (IU/L)	48.21 ± 37.78	53.13 ± 43.96	0.677
ALT (IU/L)	40.21 ± 34.76	42.23 ± 32.53	0.8396
AST/ALT ratio	1.45 ± 1.18	1.41 ± 0.86	0.8925
TSB (mg/dL)	7.39 ± 9.84	9.31 ± 10.79	0.5234

AST: Aspartate aminotransferase, ALT: Alanine transaminase, TSB: total serum bilirubin

The cutoff value of hyaluronic acid was calculated using receiver operating characteristic (ROC) test and it was found to be 1.66 ng/mL (figure 2), the sensitivity and specificity of estimation of HA value in diagnosis of liver cirrhosis were 87.1% and 94.74% respectively, while the positive predictive value of HA in diagnosis of liver cirrhosis was 96.43% and the negative predictive value was 81.82% (tables 4 and 5).

Table 4. Cutoff values, sensitivity and specificity of Hyaluronic acid in all patients groups

Cutoff value	Specificity	Sensitivity	Area under curve	P value
1.66 ng/mL	94.74%	87.1%	0.948	<0.001

Table 5. Positive and negative predictive values for Hyaluronic acid in all patients groups

Predictive values		Cirrhosis		Total
		Positive	Negative	
Hyaluronic acid	Positive test	27	1	28
	Negative test	4	18	22
	Total	31	19	50

+ve predictive value: 96.43%, -ve predictive value: 81.82%

Discussion:

The liver excretes most of circulating HA, while the kidney accounting for approximately 1%. In the liver, HA is cleared from the circulation by binding to CD44 adhesion molecules on sinusoidal endothelial cells, with subsequent transport into the hepatocyte. The CD44 is a transmembrane glycoprotein involved in the interaction between cells and extracellular matrix. Sinusoidal endothelial cells lack a basement membrane and hence, are permeable to molecules moving into hepatocytes. In the presence of fibrosis, the sinusoidal endothelial cells become thickened which is less permeable, so HA clearance is impaired and serum levels rise. Fibrosis also stimulates hepatic mesenchymal cells, so more HA is produced. Serum hyaluronic acid levels are also raised in joint inflammation, due to increased synovial

production. Xie *et al* studied the relationship between HA and procollagen III and collagen type IV and the degree of the liver fibrosis and they reported that HA had the closest correlation with fibrosis [19]. Some studies assessing HA levels have suggested serial measurements are recommended to predict clinical prognosis and correlate HA measurements with other biochemical markers of disease e.g., serum bilirubin and transaminases [20,21].

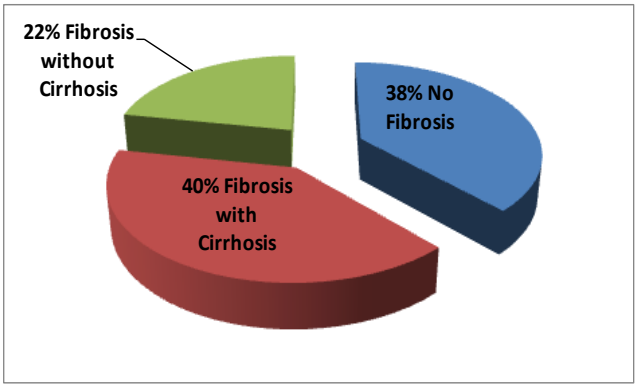

Fig. 1 Results of Liver Biopsy

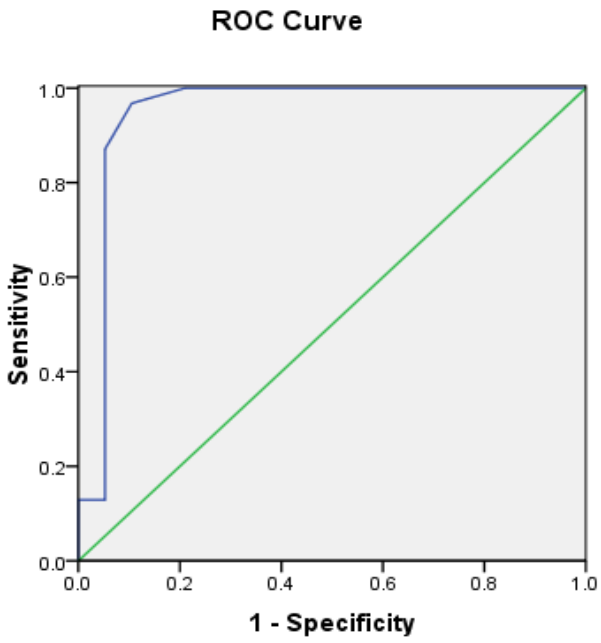

Fig. 2 The receiver operator characteristic (ROC) for sensitivity and specificity of hyaluronic acid test

In children, HA has been studied in specific etiological groups. In biliary atresia, the serum levels of HA measured at diagnosis was found to predict the need for liver transplant within the first five years [22,23]. Comparing serum HA levels with histological fibrosis in children with biliary atresia, Kobyashi *et al* [24] and Hasegawa *et al* [25] showed that significant fibrosis correlated with increased HA levels. In children with cystic fibrosis with biochemical or radiological evidence of liver disease, HA levels were also raised [15].

This study has assessed the validity of HA as a marker of fibrosis in pediatric patients with chronic liver disease, by comparing its level with the gold standard histological diagnosis of fibrosis, or with ultrasound assessment. Thirty one of 50 children with chronic liver disease had significant fibrosis, 20 of them were classified as cirrhotic liver, showed high level of HA.

The patients without significant fibrosis (mild to moderate) were reported to have a mean of HA level 1.18±0.86 ng/ml which is distinguished from those with significant fibrosis (3.05±1.11 ng/ml).

Our findings of the receiver operating characteristic (ROC) study are in agreement with those of the previous studies. Nyberg *et al* [27] have that HA levels are a sensitive tool that can be used to determine the degree of progressive liver injury in patients with primary biliary cirrhosis. Fried *et al* [28] showed that patients who developed liver fibrosis (LF) from chronic veno-occlusive diseases also presented with elevated HA levels. Other similar studies involving patients with hepatitis C also revealed a direct correlation between fibrosis in liver biopsies and elevated HA levels [29,30]. In Iran, Montazeri *et al* [31] reported a relationship between serum hyaluronate and the severity of inflammation and fibrosis in patients with non-HBsAg hepatitis B. Furthermore; the study of Hartley *et al* [32] confirmed these findings in a sample of unselected children undergoing liver biopsy.

References

1. Cohen M, A-Kader H, Lambers D, et al. Complications of percutaneous liver biopsy in children. Gastroenterology. 1992; 102: 629-32.
2. Cohen M, Cameron C, Duncan P. Pediatric anesthesia morbidity and mortality in the perioperative period. Anesth Analg. 1990; 70: 160-7.
3. Piccininno F, Sagnelli E, Pasquale G, et al. Complications following percutaneous liver biopsy. J Hepatol. 1986; 2: 165-73.
4. Terjung B, Lemnitzer I, Dumoulin FL, et al. Bleeding complications after percutaneous liver biopsy. An analysis of risk factors. Digestion. 2003; 67: 138-45.
5. The French METAVIR Cooperative Study Group. Intraobserver and interobserver variations in liver biopsy interpretation in patients with chronic hepatitis C. Hepatology. 1994; 20: 15-20.
6. Westin J, Lagging LM, WejstalR, Norkrans G, et al. Interobserver study of liver histopathology using the Ishak score in patients with chronic hepatitis C virus infection. Liver. 1999; 19: 183-7.
7. Gronbaek K, Christensen PB, Hamilton-Dutoit S, et al. Interobserver variation in interpretation of serial liver biopsies from patients with chronic hepatitis C. J Viral Hepat. 2002; 9: 443-9.
8. Bravo AA, Sheth SG, Chopra S. Liver biopsy. N Engl J Med. 2001; 344: 495-500.
9. Ishak K, Baptista A, Bianchi L, et al. Histological grading and staging of chronic hepatitis. J Hepatol 1995; 22: 696-9.
10. Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. Hepatology. 1996; 24: 289-93.
11. Joseph AEA, Saverymuttu SH, Al-Sam S, et al. Comparison of liver histology with ultrasonography in assessing diffuse parenchymal liver disease. Lin Radiol 1991; 43: 26-31.
12. Bonekamp S, Kamel I, Solga S, et al. Can imaging modalities diagnose and stage hepatic fibrosis and cirrhosis accurately. J Hepatol 2009 Jan; 50(1): 17-35.
13. Zhou K, Lu LG: Assessment of fibrosis in chronic liver diseases. J Digest Dis. 2009, 10(1): 7-14.
14. George J, Tsutsumi M, Takase S. Expression of hyaluronic acid on N-nitrosodimethylamine induced hepatic fibrosis in rats. Int J Biochem Cell Biol. 2004; 36: 307-19.
15. Wyatt HA, Dhawan A, Cheeseman P, et al. Serum hyaluronic acid concentrations are increased in cystic fibrosis patients with liver disease. Arch Dis Child. 2002; 86(3): 190-3.
16. Ding H, Chen Y, Feng X, et al. Correlation between liver fibrosis stage and serum liver fibrosis markers in patients with chronic hepatitis B. Zhonghua Gan Zang Bing Za Zhi. 2001; 9(2): 78-80.
17. Iacobellis A., Fussili S, Magnia A, et al. Ultrasonographic and biochemical parameters in the noninvasive evaluation of liver fibrosis in hepatitis C virus chronic hepatitis. Alimen Pharmacol Therap. 2005; 22(9): 769-74.
18. Ziol M, Handra-Luca A, Kettaneh A, et al. Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with chronic hepatitis C. Hepatology. 2005, 41(1): 48-54.
19. Xie S, Yao J, Zheng R, et al. Serum hyaluronic acid, procollagen type III and IV in histological diagnosis of liver fibrosis. Hepatobil Pancreat Dis Int. 2003; 2: 69-72.
20. Leroy V, Monier F, Bottari S, et al. Circulating matrix metalloproteinases 1, 2, 9 and their inhibitors TIMP-1 and TIMP-2 as serum markers of liver fibrosis in patients with chronic hepatitis C: comparison with PIIINP and hyaluronic acid. Am J Gastroenterol. 2004; 99: 271-9.
21. Lindqvist U, Chichibu K, Delpech B. Seven different assays of hyaluronan compared for clinical utility. Clin Chem. 1992; 38: 127-32.
22. Dhawan A, Trivedi P, Cheeseman P, et al. Serum hyaluronic acid as an early prognostic marker in biliary atresia. J Pediatr Surg. 2001; 36: 443-6.
23. Trivedi P, Dhawan A, Risteli J, et al. Prognostic value of serum hyaluronic acid and type I and III procollagenpropeptides in extrahepatic biliary atresia. Pediatr Res. 1995; 38: 568-73.
24. Kobyashi H, Horikoshi K, Yamataka A, et al. Hyaluronic acid: a specific prognostic indicator of hepatic damage in biliary atresia. J Pediatr Surg. 1999; 34: 1791-94.
25. Hasegawa T, Tsasaki S, Kimura T, et al. Measurement of serum hyaluronic acid as a sensitive marker of liver fibrosis in Biliary atresia. J Pediatr Surg. 2000; 35: 1643-6.
26. Hosmer DW, Lemeshow S. Applied Logistic Regression. 2nd ed. USA: John Wiley & Sons Inc; 2000. p. 156-64.
27. Nyberg A, Engstrom-Laurent A, Loof L. Serum hyaluronate in primary biliary cirrhosis--a biochemical marker for progressive liver damage. Hepatology. 1988; 8(1): 142-6.
28. Fried MW, Duncan A, Soroka S, et al. Serum hyaluronic acid in patients with veno-occlusive disease following bone marrow transplantation. Bone Marrow Transplant. 2001; 27(6): 635-9.
29. Halfon P, Bourliere M, Penaranda G, et al. Accuracy of hyaluronic acid level for predicting liver fibrosis stages in patients with hepatitis C virus. Comp Hepatol. 2005; 4: 6.
30. Skripenova S, Trainer TD, Krawitt EL, et al. Variability of grade and stage in simultaneous paired liver biopsies in patients with hepatitis C. J Clin Pathol. 2007; 60(3): 321-4.
31. Montazeri G, Estakhri A, Mohamadnejad M, et al. Serum hyaluronate as a non-invasive marker of hepatic fibrosis and inflammation in HBeAg-negative chronic hepatitis B. BMC Gastroenterol. 2005; 5: 32.
32. Hartley JL, Brown RM, Tybulewicz A, et al. Hyaluronic acid predicts hepatic fibrosis in children with hepatic disease. J Pediatr Gastroenterol Nutr. 2006; 43: 217-21.
33. Pradat P, Alberti A, Poynard T et al. Predictive value of ALT levels for histologic findings in chronic hepatitis C: a European Collaborative Study. Hepatology. 2002; 36: 973-7.

Correspondence to Nadia N Hassan
E-mail: nadiacom72@yahoo.com
Phone: + 964 7901273878
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