Hyaluronic Acid Predicts Hepatic Fibrosis in Children With Hepatic Disease

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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.

Methods: Ninety-three unselected consecutive children (median age, 7.5 years; range, 0.07–19 years) undergoing a liver biopsy between April 2003 and March 2004 were prospectively recruited. Liver biopsy and fasting HA levels were taken simultaneously. The Ishak score was used to stage fibrosis. Scores of 3 or greater were regarded as significant fibrosis. Hyaluronic acid levels were measured using an enzyme-linked binding protein assay (2002 Corgenix, Inc) (adult reference range, 0–75 ng/mL; pediatric reference range, 0–30 ng/mL).

Results: Twenty-three (25%) of 93 biopsies had significant fibrosis, and HA levels in this group were significantly higher than those with mild fibrosis (Ishak score, <3), (median level, 72 ng/mL vs 30 ng/mL; Mann-Whitney U test; P < 0.005). Hyaluronic acid level of 50 ng/mL had a positive predictive value 40% and negative predictive value 86% for significant fibrosis. An HA level 200 ng/mL has a sensitivity of 26% and specificity of 90%.

Conclusions: Hyaluronic Acid is a valid noninvasive predictor of hepatic fibrosis in unselected children with liver disease. An HA level of 200 ng/mL strongly suggests significant fibrosis. Hyaluronic acid level of less than 50 ng/mL accurately identifies those who do not have significant fibrosis.

Key Words: Hyaluronic acid—Sinusoidal cell adhesion molecules—Fibrosis—Ishak score—Pediatric liver disease. © 2006 Lippincott Williams & Wilkins

INTRODUCTION

Many children each year require a liver biopsy under anesthesia. The precise indications for biopsy are diverse, but in all these cases, histopathologic examination is essential to guide clinical management.

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). Philadelphia Serious complications include hemoperitoneum, pneumothorax and bile leak (3). Approximately 1% of liver biopsies are associated with these complications (4), and therefore, liver biopsies are only carried out if deemed essential.

Liver biopsies have been regarded as the gold standard for assessment of fibrosis, but the limitations of the technique have to be taken into account. Fibrosis is not always uniform throughout the liver. A study of laparoscopic biopsies taken of both the left and right lobes of the liver showed that cirrhosis was diagnosed in 1 lobe only in 14% of cases (5). When 3 liver biopsies were taken at the same time, only 50% of cases had cirrhosis identified in all 3 biopsies (6). Histological comparison of liver biopsies is limited by the lack of an ideal hepatic fibrosis scoring system covering all spectrums of liver disease. Scores have been developed for chronic hepatitis such as the Scheuer score and the Histological Activity Index of Knowdell. The Ishak scoring system has been developed from the Histological Activity Index of Knowdell with modifications allowing for increased knowledge of etiology and histology (7). Rosenberg et al. (8) used both the Scheuer score and the Ishak score and found similar results. An accurate, noninvasive marker for liver fibrosis could reduce the need for liver biopsy and would be a preferable method for serial monitoring of disease progression in order to titrate treatment when necessary (9).

Hyaluronic acid is a high molecular weight glycosaminoglycan synthesized by mesenchymal cells, circulated by the lymphatic system and widely distributed in connective tissue (10). It has a structural role in connective tissue matrix and is involved in interactions between cells. It has a half-life of 5 to 6 minutes in plasma. It is
found in high concentrations in the synovium, where its role is to retain water and lubricate the joint. The liver excretes most of HA, with 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. 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 into a vascular type of endothelium, 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.

Previous studies have looked at a range of large molecular weight proteins involved in connective tissue to identify those which best correlate with the degree of liver fibrosis. When comparing HA with procollagen III and collagen type IV, HA had the closest correlation with fibrosis (11). Further studies evaluating HA to determine its diagnostic validity in pediatric liver disease are therefore warranted.

Studies assessing HA levels have used serial measurements to predict clinical prognosis and correlate HA measurements with other biochemical markers of disease (e.g., serum bilirubin and transaminases) (12,13). No studies to date have correlated HA with histological staging of hepatic fibrosis in unselected pediatric patients.

AIM

The aim of the study was to determine the validity of serum HA as a marker of hepatic fibrosis in an unselected group of children with liver disease, undergoing a liver biopsy.

MATERIALS AND METHODS

This is a prospective cohort study of unselected children undergoing liver biopsy for clinical indications. Ninety-three consecutive children undergoing liver biopsy at Birmingham Children’s Hospital between April 2003 and March 2004 were recruited. No children were known at the time of biopsy to have active juvenile idiopathic arthritis or any joint inflammation.

Anthropometric measurements and conventional laboratory tests of liver function were undertaken within 48 hours before the liver biopsy (Table 1 for laboratory results). From these blood results and height, a calculated glomerular filtration rate (cGFR) was formed using the Schwartz formula (height [cm] × 40/ serum creatinine [mmol/L] in those greater than 1 year of age or height [cm] × 20/serum creatinine [mmol/L] in those less than 1 year). Ninety-three liver biopsy specimens were taken with simultaneous blood for HA measurement. All the children had been fasted for a minimum of 6 hours before the sample being taken, and all the samples were taken in the morning. No child was hypoglycemic. Blood samples were separated and frozen at −20°C before analysis, which was carried out in batches at the Hepatology Unit, Chancellor’s Building, University of Edinburgh. The laboratory staff undertaking the assay were blinded to the degree of fibrosis on the biopsy and also to the clinical diagnosis. Hyaluronic acid levels were measured using an enzyme-linked binding protein assay of serum samples (2002, Corgenix, Inc) (14).

A single experienced pediatric 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. The liver biopsy specimens were staged for fibrosis using the Ishak scoring system (7), with a maximum score of 6. A deviation from the Ishak scoring system was the score of 1 given for those with pericellular fibrosis. Significant fibrosis was taken as an Ishak score of 3 or more.

A preliminary study to identify a reference range for HA in healthy children (those without liver disease) was conducted. Forty children (median age, 7.7 years; range, 1.6–14.9 years; 20 boys) in the Royal Hospital for Sick Children, Edinburgh, were fasted for a minimum of 4 hours before minor surgery. The blood samples were taken during the anesthetic and processed as previously described. The median HA level was 0 ng/mL (range, 0–29.2 ng/mL). This suggests that the normal value for HA in healthy children is less than 30 g/mL.

The South Birmingham Research and Ethics committee and the Lothian Regional Ethics committee granted ethical approval. Both an age-appropriate written and verbal explanations of the study were given to the child and parents. Written consent was obtained for the liver biopsy and blood specimen.

STATISTICS

All the data were entered onto an Statistical Package for the Social Sciences (SPSS Inc, Chicago, IL) file for analysis. To create a normal distribution, HA was converted into log HA for analysis. The Mann-Whitney U test was used to compare HA levels in those with and without significant fibrosis. Logistic regression analysis

TABLE 1. Biochemical and liver function results

<table>
<thead>
<tr>
<th>Test</th>
<th>Median</th>
<th>Minimum</th>
<th>Maximum</th>
<th>25th Percentile</th>
<th>75th Percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total bilirubin, μmol/L (0–24 μmol/L)</td>
<td>11</td>
<td>5</td>
<td>550</td>
<td>5</td>
<td>22</td>
</tr>
<tr>
<td>Alkaline phosphatase, IU/L (125–875 IU/L)</td>
<td>444</td>
<td>122</td>
<td>1963</td>
<td>333</td>
<td>743</td>
</tr>
<tr>
<td>Alanine transferase, IU/L (10–55 IU/L)</td>
<td>62</td>
<td>10</td>
<td>1566</td>
<td>26</td>
<td>106</td>
</tr>
<tr>
<td>Aspartate transferase, IU/L (15–40 IU/L)</td>
<td>64</td>
<td>24</td>
<td>2255</td>
<td>39</td>
<td>128</td>
</tr>
<tr>
<td>γ-Glutamyltransferase, IU/L (0–35 IU/L)</td>
<td>51</td>
<td>13</td>
<td>2000</td>
<td>22</td>
<td>130</td>
</tr>
<tr>
<td>Albumin, g/L (37–56 g/L)</td>
<td>39</td>
<td>24</td>
<td>48</td>
<td>34</td>
<td>42</td>
</tr>
<tr>
<td>Prothrombin time, s (9–13 s)</td>
<td>12</td>
<td>10</td>
<td>18</td>
<td>11</td>
<td>12</td>
</tr>
</tbody>
</table>

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was undertaken with the presence and absence of significant fibrosis as the dependent variable and log HA levels and standard liver function tests (prothrombin time, platelet count, serum bilirubin, serum albumin, γ-glutamyltransferase, alanine transferase and aspartate transaminase) as independent variables. To assess clinical applicability, a receiver operator curve was constructed and the area under the curve calculated using Statistical Package for the Social Sciences.

RESULTS

Ninety-three paired liver biopsy and serum HA samples were collected. The median age at biopsy was 7.5 years (range, 0.07–19 years), 41% girls and 59% boys. Forty children had undergone a liver transplant, and therefore, the biopsy was of the allograft.

The underlying clinical diagnoses and histological findings for those children posttransplantation are summarized in Table 2.

There was no correlation between the cGFR and the log HA level ($P = 0.56$). One child had a cGFR of 57 mL/min per 1.73 m$^2$.

Significant fibrosis was found in 23 of 93 (25%) biopsies with 5 of these biopsies classified as cirrhotic (Ishak stage 6).

The HA levels in those with significant fibrosis (Ishak score, $\geq 3$) was significantly higher than the children with no or mild fibrosis (Ishak score, $<3$) (median level, 72 ng/mL [range, 4.3–1077.6 ng/mL] vs 30 ng/mL [range, 4.32–1022.5 ng/mL], Mann-Whitney $U$ test $P < 0.005$). Using univariate logistic regression analysis of all the biochemical measurements, log HA and serum albumin were the only variables predictive of significant fibrosis. On stepwise logistic regression analysis, log HA was the only significant variable predictive of hepatic fibrosis ($\chi^2, P = 0.007$) (Table 3).

The area under the curve of the receiver operator curve for HA levels is 0.69 (SE, 0.66; $P = 0.006$) (Fig. 1).

Hyaluronic acid level of 50 ng/mL had a sensitivity of 65%, specificity of 68%, positive predictive value of 40% and negative predictive value of 86%, for significant fibrosis. Sensitivity was greatly reduced with higher cutoff values (HA level of 200 ng/mL has a sensitivity of 26%). Hyaluronic acid level of 200 ng/mL was strongly diagnostic of significant fibrosis with a specificity of 90% (Table 4). An HA level of 200 ng/mL had a positive predictive value 46% and negative predictive value 78%.

DISCUSSION

This is the first study to show the validity of HA as a marker of fibrosis in unselected pediatric patients with liver disease, by comparing with the gold standard histological diagnosis of fibrosis, in a blinded study.
Hyaluronic acid levels were significantly increased in those with significant fibrosis. This was despite a cohort of patients with mild disease, with only 25% having significant fibrosis (5 with cirrhosis). In previous studies of both adults and children, the patients were known to have chronic liver disease, and therefore, a larger portion of the cohort had significant fibrosis. In our study, the patients undergoing biopsies were unselected for etiology and had mild disease. This will have impacted on the statistical significance of the results and influenced the outcome of this study.

Hyaluronic acid is highly specific at levels of 200 ng/mL, indicating that there is a strong likelihood of the child having significant fibrosis. A number of children with significant fibrosis however would not have been identified as the sensitivity of the test is low. A cohort of patients with more severe liver disease may allow the calculation of a suitable HA cutoff level to provide a clinically acceptable sensitivity and specificity to diagnose significant fibrosis.

The clinical application from this study is in the ability to identify those children who do not have significant fibrosis. An HA level of 50 ng/mL has an excellent negative predictive value of 86%. Children with milder disease can therefore be identified.

In adults, the normal value for HA has been determined as 0 to 75 ng/mL (13). In pediatrics, a preliminary study of 40 children without liver disease (median age, 7.7 years; range, 1.6–14.0 years; 20 girls) had serum levels taken in a fasted state during minor surgery. Using the same enzyme-linked binding protein assay as in this study, the results suggested the reference range to be 0 to 30 ng/mL (median, 0.0; range, 0.0–29.3 ng/mL) (Tybulewicz A, Gillett PM, Wilson DC et al. HA levels in childhood liver disease—defining a normal range. Unpublished data, 2003). Although there have been numerous studies using HA in adult liver disease, the results cannot be directly applied to children.

In this study, the Ishak scoring system was used to quantify liver histology. The degree of fibrosis is a continuum, but to enable comparison between patients, a discontinuous semiquantitative score is assigned from 0 to 6. The Ishak scoring system was designed and validated for use in chronic hepatitis. Although the pediatric population studied was unselected in most cases, the Ishak scoring system seemed appropriate because they had features in keeping with chronic hepatitis. This includes the posttransplant patients who underwent routine postsurveillance biopsies and were found to have chronic hepatitis. Not all the patients had chronic liver disease, but for constancy, the Ishak score was used consistently throughout the study because there is no single scoring system for all etiologies.

In an adult study of patients with chronic liver disease, serum HA was shown to be a sensitive and specific indicator of cirrhosis (at a cutoff of 100 ng/mL, HA had a sensitivity of 83% and specificity of 78%) (9). The sensitivity and specificity was different for specific disease groups with primary biliary cirrhosis having the best results (sensitivity 93% and specificity 100% with a HA level cutoff of 100 ng/mL). In adults with non-alcoholic fatty liver disease, HA was able to distinguish between increasing severity of fibrosis (a cutoff of 46.1 μg/L had a sensitivity of 85%, specificity of 80%, positive predictive value of 51% and negative predictive value of 96%) (15). We presume these differences reflect differing populations and varying pathogenesis.

In children, HA has been studied in specific etiological groups. In biliary atresia, HA measured at diagnosis was found to predict the need for liver transplant within the first 5 years (16,17). Comparing HA with histological fibrosis in children with biliary atresia (18,19) showed similar findings to those of our study. Significant fibrosis correlated with increased HA levels. In children with cystic fibrosis (20) with biochemical or radiological evidence of liver disease, HA levels are also raised. No studies have looked at histological fibrosis in this cohort of patients.

Both animal models and human studies have shown that serum HA increases in the presence of hepatic fibrosis (9). In attempting to identify a noninvasive marker of fibrosis, other extracellular matrix molecules have been studied and are increased in fibrosis, but these have not been as sensitive as HA. In our study, liver function tests and measures of hepatic synthetic function, other than serum albumin, did not correlate with the histological stage of fibrosis. In adults, collagen type III and collagen type IV showed that they had a reduced sensitivity and specificity of diagnosing cirrhosis as compared with serum hyaluronic acid. However, when studying adults with hepatitis C (12), the combination of procollagen III N-terminal peptide and matrix metalloproteinases 1, had a greater sensitivity and specificity for cirrhosis than hyaluronic acid alone or in combination. In children with biliary atresia hyaluronic acid, type III procollagen and type I procollagen have been assessed for their prognostic value. Only HA (16) was shown to be of any benefit in predicting those who were likely to die without transplant.

A histologically controlled study of chronic liver disease in adults using a panel of serum fibrosis markers (including HA) found a sensitivity of 90% and negative

### Table 4

<table>
<thead>
<tr>
<th>HA level (ng/mL)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Positive predictive value (%)</th>
<th>Negative predictive value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;50</td>
<td>65</td>
<td>68</td>
<td>40</td>
<td>86</td>
</tr>
<tr>
<td>&gt;100</td>
<td>52</td>
<td>74</td>
<td>36</td>
<td>79</td>
</tr>
<tr>
<td>&gt;150</td>
<td>39</td>
<td>77</td>
<td>45</td>
<td>80</td>
</tr>
<tr>
<td>&gt;200</td>
<td>26</td>
<td>90</td>
<td>46</td>
<td>78</td>
</tr>
</tbody>
</table>

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predictive value of 92% for the detection of significant fibrosis. The area under the receiver operator curve was 0.804. This panel of markers provides a clinically useful tool to measure fibrosis (8).

The area under the receiver operator curve for this current study was 0.69. This is statistically significant; however, in clinical practice, it is recommended that a test has an area under the curve of 0.8 or greater to be clinically useful.

In the future, we aim to evaluate the use a panel of noninvasive markers including HA, in groups with more severe liver disease, in selected etiological groups and to evaluate serial measurements in defined patient populations.

CONCLUSIONS

Hyaluronic acid is a valid noninvasive predictor of histological fibrosis in unselected children with liver disease. A high level indicates that fibrosis is likely, and a low level can aid the identification of those with mild fibrosis. It complements the thorough investigation of a child with liver disease but cannot at present replace histological examination to identify fibrosis. Further evaluation of HA is needed to ascertain the use of serial measurements in targeted patient groups.

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