ORIGINAL ARTICLE

Serum hyaluronic acid concentrations are increased in cystic fibrosis patients with liver disease

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Aim: To determine whether serum hyaluronic acid (HA) concentrations are abnormal in patients with cystic fibrosis (CF) liver disease, and if so, whether the abnormality is associated with disease severity. **Methods:** A total of 74 patients with CF were assessed for evidence of liver involvement as indicated by clinical, ultrasound, and biochemical findings. Serum hyaluronic acid concentrations were measured and compared with concentrations in 293 normal controls. Lung function in the CF patients was also recorded.

Results: Thirty four CF patients had no evidence of liver disease; in these, serum HA concentrations were similar to those in healthy controls (median (range): 16.1 (9.4-75.1) v 15 (1-77) µg/l). Nineteen CF patients had established liver disease detected by clinical and ultrasound examination, with significantly increased HA concentrations (56.1 (26-355) µg/l). Serum HA concentrations were also significantly increased, although to a lesser extent, in 21 CF patients with an abnormal liver ultrasound scan alone (22.4 (9.5-43.4) µg/l). There was no correlation between serum HA concentration and lung function.

Conclusion: Serum HA concentrations were significantly increased in children with clinical or ultrasound evidence of liver disease, being higher in those with more advanced hepatic damage. Despite the inflammation and fibrosis present in CF lungs there was no correlation between HA concentration and lung function, suggesting that high concentrations were a failure of hepatic clearance rather than overproduction in the lung. Longitudinal measurement of HA concentrations may prove a useful marker for the development of significant liver damage in CF patients.

Liver involvement in cystic fibrosis (CF) is frequent, but clinically overt liver disease is uncommon although associated with significant morbidity and mortality.^{1,2} The pathological process of focal biliary fibrosis progressing to cirrhosis and portal hypertension has been well described, particularly with relation to the increased understanding of the role of the CF transmembrane conductance regulator in the biliary epithelium.^{1,2} The true prevalence of liver involvement is unclear, however, varying from 2% to 35% depending on the definition used and the patient age group studied.³⁻⁶ As the overall survival in CF continues to increase, older patients with previous subclinical liver involvement may be vulnerable to progression to significant liver disease. Currently it is not possible to predict those most at risk of developing complications of liver involvement, and this in turn hampers the evaluation of potential disease modifying therapies used early in the course of the process. Clinical evidence of hepatic fibrosis is generally only apparent when the disease has become well established and the changes irreversible. Hepatocyte function is usually well preserved in CF liver disease until the process is advanced. Most commonly used markers of hepatic disease, however, are a measure of hepatocyte dysfunction rather than fibrosis, and as such are unhelpful in the early stages of liver disease in CF. In many non-CF liver diseases the diagnosis rests on histological findings; however in CF the patchy distribution of biliary involvement limits the value of liver biopsy. Other modalities, such as ultrasound (US) scanning and hepatobiliary scintigraphy are specific in the detection of abnormalities of the liver parenchyma and the biliary structure, but have not been shown to have prognostic value.^{7,8}

A feature of CF related liver disease is progressive biliary fibrosis. A potentially helpful marker of liver function in this condition could be hyaluronic acid (HA), a glycosaminoglycan rapidly cleared from the circulation by the liver, via receptor

mediated endocytosis into sinusoidal endothelial cells.^{9,10} Besides impairment of endothelial cell function secondary to liver disease, raised serum HA could also be caused by its release into the circulation as a result of increased synthesis in the liver during hepatic fibrogenesis.¹¹ The aim of this study was to determine whether concentrations of serum HA are abnormal in patients with CF liver disease, and if so, whether the abnormality is associated with liver disease severity.

PATIENTS AND METHODS

Subjects were recruited from both the regional CF centre and the supraregional hepatology service at King's College Hospital, London. The diagnosis of cystic fibrosis had been previously established by increased sweat chloride and/or the presence of two known CF gene mutations. Patients were enrolled sequentially over a four year period when they attended either for their routine annual review or for a specialist liver appointment. Assessment included clinical examination, lung function testing, and an abdominal US scan. Biochemical tests of liver function were performed, including serum transaminases, γ glutaryl transferase, and international normalised prothrombin ratio (INR). A serum sample for HA concentration was stored at -70°C for later analysis. The subjects were divided into three groups according to clinical and US findings. Group 1 had no clinical, biochemical, or US evidence of hepatic abnormality. Group 2 comprised those with no abnormal clinical or biochemical findings, but a US scan with heterogenous and nodular liver

Abbreviations: CF, cystic fibrosis; HA, hyaluronic acid; HAPB, HA binding protein; INR, international normalised prothrombin ratio; UDCA, ursodeoxycholic acid; US, ultrasound

Table 1 Results of liver biochemistry, serum HA, and lung function in CF patients according to degree of liver involvement

	Normal controls	Group 1	Group 2	Group 3
No.	293	33	22	19
Age (y)	10.4 (3.5)	10.1 (4.2)	11.5 (3.0)	14.8 (3.8)
Sex (M:F)	0.9	2.0	1.4	2.2
γ glutaryl transferase (IU/l)	–	10.7 (4.2)	21.3 (11.3)	83.6 (103.3)
Aspartate transaminase (IU/l)	–	25.5 (7.8)	30.9 (8.3)	50.9 (26.4)
Alkaline phosphatase (IU/l)	–	214.1 (67.4)	263.1 (57.4)	367 (191.9)
Bilirubin (μ mol/l)	–	5.5 (2.8)	6.1 (3.6)	15.7 (13.1)
INR	–	1.0 (0.2)	1.0 (0.09)	1.18 (0.18)
HA (μ g/l)	15 (1–77)	15.9 (9.4–75.1)	23.2 (9.5–43.4)	56.1 (26–355)
p value	–	>0.05	<0.05	<0.001
No.	–	33	19	14
FEV ₁ (% predicted)	–	74.2 (24.4)	77.1 (23.7)	70.9 (28.9)
FVC (% predicted)	–	77.5 (20.1)	78.2 (18.6)	78 (27.3)

Results expressed as mean (SD) except for HA level which is median (range). Probability values are for serum HA levels; each group is compared to normal controls using the Kruskal–Wallis test.

parenchyma. Group 3 had clinical evidence of liver disease, and a US scan which showed a heterogenous and nodular parenchyma, and hepatosplenomegaly, with or without portal hypertension.

Clinical liver disease was defined as the presence of a firm liver edge palpable more than 2 cm below the costal margin in the right mid-clavicular line, or a palpable left lobe of the liver, and/or splenomegaly. Portal hypertension or ascites were also considered indicative of established hepatic disease. The liver US examination assessed the size, overall parenchymal reflectivity, the attenuation, and the parenchymal echo pattern. Assessment of parenchymal echo pattern is subjective and was therefore only performed by one of two ultrasonographers who had extensive experience in this area. US was also used to assess the biliary tract for evidence of obstruction or gall bladder disease, and to measure the size of the spleen. The maximum splenic length was recorded as the longest length from three measurements, and compared with recognised, age related values. Abnormal liver function tests were deemed present if greater than twice the upper limit of normal for our laboratory (γ glutaryl transferase 55 IU/l; aspartate aminotransferase 50 IU/l; total bilirubin 20 μ mol/l; alkaline phosphatase, age and sex related ranges used) and INR greater than 1.2. Seventy five CF patients were eligible for the study. According to the above criteria, 33 of them had no evidence of liver disease (group 1), 22 had an abnormal US scan alone (group 2), and 19 had clinical and US evidence of established liver disease (group 3). Only one patient had elevation of liver function tests in the absence of detectable clinical or US abnormality. This patient was excluded from the study as the derangement in liver function tests was transient and considered to be secondary to ciprofloxacin therapy.

Lung function was measured by spirometry and expressed as a percentage of that predicted for age, sex, and height.¹² Measurements were usually done at the time of the assessment, and only spirometry recorded within two months from when blood was taken for HA concentration was included.

HA was measured using an affinity binding radiometric assay (Pharmacia Diagnostics, Uppsala, Sweden) which uses an HA binding protein (HABP) prepared from bovine cartilage. The HA in 100 μ l aliquots of undiluted serum bound to ¹²⁵I-labelled specific HABP. Free ¹²⁵I-HABP was then bound to exogenously added HA covalently linked to Sepharose and the complex separated by centrifugation. The amount of bound radioactivity was measured in a gamma counter and the response was inversely proportional to the concentration of HA in the sample. A normal range for hyaluronic acid had been determined previously by our group from a large group of healthy children and adolescents, aged from 5 to 19 years.¹³

Statistics

Normally distributed data were analysed using one way ANOVA with Bonnferroni correction for multiple comparisons. Serum HA concentrations were not normally distributed and were therefore analysed using the non-parametric Kruskal–Wallis test with Dunn's test for multiple comparisons.

RESULTS

The healthy control population and the CF patients who had either a normal US scan (group 1) or an abnormal US scan

Figure 1 Serum HA concentrations in normal controls and CF patients according to degree of liver involvement. Median values are indicated by a horizontal bar.

alone (group 2) were of a similar age. The patients with established liver disease (group 3) were significantly older than the other two CF patient groups ($p \leq 0.05$) (table 1).

Where lung function was recorded (14/19), the patients with liver disease had slightly poorer FEV₁ than those without, but this was not statistically significant. There was no correlation between lung function and HA concentration in the CF patients ($r^2 = 0.0001$). Standard liver function tests in groups 1 and 2 were all within their normal ranges. Although the liver function tests tended to be higher in group 2 compared to group 1, this was not statistically significant. Aspartate transaminase, γ glutaryl transferase, INR, bilirubin, and alkaline phosphatase were all significantly higher in group 3 than either group 1 or group 2.

Healthy subjects and CF patients with no evidence of liver disease (group 1) had similar HA concentrations, while concentrations were significantly increased in groups 2 and 3 compared to the normal controls (fig 1). Serum HA concentrations were also significantly different between groups 2 and 3 ($p < 0.005$), although the difference between groups 1 and 2 did not reach significance.

DISCUSSION

Standard tests of liver function are unhelpful in monitoring the liver involvement in CF.⁵ A reliable and minimally invasive method of detecting the presence and severity of hepatic fibrosis in CF is needed for the improved management of this condition. It would enable a more accurate assessment of the current prevalence of liver disease than previous estimates have achieved, and would also track any changes in the natural history of the process that may occur with the improving survival in CF. The early detection of hepatic fibrosis would predict those at risk of clinically significant liver disease who might potentially benefit from therapeutic intervention. The benefits of such intervention may be greater if commenced in the early stages of the disease process, but further clinical trials of disease modifying drugs require an accurate and dynamic marker of fibrosis.

Our cross sectional study suggests that serum HA concentrations in CF patients reflect the degree of hepatic damage. Serum HA concentrations were significantly increased in children with clinical liver disease. They were also significantly increased, although to a lesser extent, in a group of children with heterogeneous or nodular changes seen on liver ultrasound scan. CF patients without either clinical or US evidence of liver involvement (group 1) had HA concentrations similar to healthy controls.

HA is one of the glycosaminoglycans; it is found in all tissues and body fluids but is particularly abundant in loose connective tissue.^{14 15} It is a vital component of the viscoelasticity of extracellular matrix and contributes to lubrication between tissues. It is synthesised in the plasma membrane of fibroblasts and other cells, primarily in connective tissue and synovial membranes. Some HA is metabolised locally, but most is transported by lymphatic pathways to blood, from where over 90% is cleared rapidly by the liver via receptor facilitated uptake and catabolism in liver endothelial cells.^{9 10 14} The half life of HA in the circulation of healthy subjects is 2–5 minutes.^{10 15} Normal serum HA concentrations are age dependent with a decline from infancy to adulthood, and rise again in the elderly.^{13 16} Raised serum HA concentrations have been shown in non-CF liver diseases and are considered to be due largely to impaired clearance of HA from the circulation by damaged liver endothelial cells.^{9 10 14 15} More than 10 years ago Nyberg and colleagues showed that serum hyaluronate is a sensitive marker for progressive liver damage in primary biliary cirrhosis.¹⁷ However, despite its potential usefulness serum HA has not become widely used in primary biliary cirrhosis because it does not offer more accurate information than the simple measurement of bilirubin concentrations in

this condition.¹⁸ HA concentrations are raised in the majority of infants presenting before the age of 6 months with biliary atresia, α_1 antitrypsin deficiency, and cryptogenic hepatitis and appear to be a better predictor of progressive liver disease in infancy than standard laboratory tests of liver function.¹³ We have shown previously that a raised HA before portoenterostomy in children with extrahepatic biliary atresia has a positive predictive value of 74% for identifying those patients who would die or require liver transplant before the age of 5 years.^{19 20}

All CF patients in this study were on standard medication for management of this condition, primarily comprising pancreatic enzyme replacement, multivitamin preparations, and a variable requirement for antibiotics. There is no evidence that any of these drugs affect serum HA concentrations. In addition, most of group 3 and over half of group 2 had been prescribed ursodeoxycholic acid (UDCA) prior to the start of the study. This reflected the prevailing trend in the management of CF related liver disease consequent on the reported benefit of UDCA.^{21 22} It would now be extremely difficult to obtain a group of CF patients with liver involvement who are UDCA naïve. Only one study has examined the effect of UDCA on serum HA concentrations, and this was in a group of patients with primary biliary cirrhosis. They found no significant change in serum HA concentrations associated with UDCA usage in the various histological stages of primary biliary cirrhosis.²³ It therefore seems unlikely that the use of UDCA was the cause of the increased HA concentrations that we found in groups 2 and 3. UDCA has, however, been shown to reduce raised standard liver function tests in patients with established CF related liver disease.^{21 22} Most of the patients in group 2 who were taking UDCA had normal liver function tests prior to commencement of this therapy.

Synthesis of HA also occurs in synovial membranes and raised serum HA concentrations have been found in inflammatory joint conditions. None of the CF patients had any clinical evidence of arthropathy. By chance there were twice as many boys as girls in group 1, and the greater frequency of boys in group 3 is likely to reflect the higher prevalence of liver disease in CF males generally.⁵ Previous studies, however, have shown no sex difference in serum HA concentrations.^{13 16} The patients with CF related liver disease (group 3) were significantly older than the healthy control subjects. A subanalysis of healthy controls aged 13–19 years, found a similar mean age (14.99 years) to group 3, and a slightly lower median HA concentration (14 $\mu\text{g/l}$). This further highlights the significant difference in HA concentrations between these two groups.

Serum HA concentrations in CF patients were unrelated to lung function, suggesting that the raised hyaluronate reflected hepatic disease rather than pulmonary fibrosis. This is of some surprise considering the extensive inflammation and scarring that is characteristic of CF lung disease. Increased concentrations of HA in bronchoalveolar lavage fluid have been detected in a number of other pulmonary conditions,^{24 25} and similarly raised concentrations have been found in CF lungs.²⁶ Where they have been compared, however, non-CF lung diseases with high bronchoalveolar lavage fluid HA concentrations have normal serum HA concentrations.^{24 25} Similarly, serum concentrations do not appear to reliably reflect high pulmonary concentrations of other inflammatory markers, such as interleukin 8, in CF.²⁷ It is therefore perhaps not unreasonable to conclude that the high serum HA concentrations in our study group were primarily a result of delayed clearance by the liver rather than spillover from any increased production in the lungs.

In conclusion, we have shown that serum HA is significantly increased in CF patients with clinical or US evidence of liver disease. The considerable overlap in HA concentrations between patients with no clinical or US evidence of liver disease (group 1) and those with US abnormalities (group 2)

may derive from the fact that US is a relatively insensitive test for parenchymal damage or fibrosis. It is conceivable that some of the patients with high HA concentrations in group I have liver damage not yet detectable by US. A longitudinal study of serum HA concentrations, paired with US examination, from early childhood in a large number of patients with CF would determine whether serum HA is a reliable measure of progression of hepatic fibrosis in this disease. HA in turn may prove useful in the evaluation of pharmacological treatments aimed at arresting this progression.

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