Hyaluronic acid as Serum Marker of Hepatic Fibrosis in Children chronically infected with Hepatitis C virus

Aisha Sehari [1], Mohamed A.Dekna [1], Sana H. Barakat[2], Mona A. Salem[2], Wessam M. El- Gendy[2]

[1]Tripoli University, Faculty of Medicine,Tripoli, Libya

[2]University of Alexandria, Faculty of medicine, Egypt

Abstract

Chronic hepatitis C is one of the major causes of liver disease throughout the world; nearly 170 million individuals are affected with the highest prevalence in Egypt.

The course and outcome of HCV infection is highly variable, form silent disease to development of cirrhosis and end stage liver disease. Most children with HCV progress to chronic HCV infection.

Assessment of hepatic fibrosis is important for determining prognosis, guiding management decisions, and monitoring disease. Histological evaluation of liver biopsy is currently considered the reference test for staging hepatic fibrosis. Since liver biopsy carries a small but significant risk; non-invasive methods to assess hepatic fibrosis are desirable.

Among the non-invasive methods: serum markers, models and imaging which are easy to perform. Serum markers of fibrosis include, direct markers (ECM) proteins which reflect balance between fibrogenesis and fibrolysis turnover and indirect markers, which reflect alterations in hepatic function.

In the present study our aim is to evaluate the diagnostic utility of hyaluronic acid (HA), Transforming Growth Factor Beta one (TGF-β1) in detecting the stage of fibrosis in children chronically infected with HCV.

To achieve this goal, 60 chronically HCV infected children, attndding EL-Shatby Alexandria University Hospital folow up clinic and 25 healthy children were included in this study. All children were evaluated clinically. CBC, Liver profile, renal functions tests, Cholesterol, Hyaluronic acid(HA), Transforming Growth factor Beta one(TGF-β1) and Ultrasound(U/S) were done to all children (cases and control).Percutaneous ultrasound guided liver biopsies were performed and classified according to Ishak scoring system in HCV infected children only.

HCV studied children were divided into 2 groups; non-significant fibrosis (stages 0/6, 1/6, 2/6) and significant fibrosis (stages 3/6 and 4/6).

Statistical analysis of data obtained from the present study showed the following results:

1-Only AST and ALT were higher in HCV children than control with significant difference

2-By using cut-off value of 24µg/L one could predict absence or presence of significant fibrosis. Significant fibrosis can be predicted by a HA level of < 24µg/l for its presence (PPV of 27.3%) and of <24µg/l for its absence (NPV of 100), with AUC of 0.747.

1- A marginal correlation was reported between TGF-β1 and degree of neco-inflammation but no correlation between TGF-β1 and different stages of fibrosis in both non-significant and significant groups.

2- Right oblique diameter of the right lobe of the liver had the best diagnostic accuracy in differentiating between significant and non-significant fibrosis.

Discussion

Chronic HCV is a progressive disease. The prognosis for patients with this disease depends largely on the development of liver fibrosis which may progress to cirrhosis; liver damage has to be regularly evaluated. The assessment of liver fibrosis provides useful information not only for diagnosis but also for therapeutic decision. Liver biopsy which has been considered as a "gold standard" tool for diagnosing and evaluation of the liver fibrosis is sometimes associated with severe complications, and for ethical reasons cannot be repeated to monitor liver status (73). Furthermore A recent study compared percutaneous biopsy with laparoscopic biopsy demonstrated that cirrhosis was missed in almost 30% of cases by the percutaneous liver biopsy. So the potentiality for error in staging hepatic fibrosis may be high that even cirrhosis can be missed in 30% of patients. (74, 75)

The improvement of the diagnostic accuracy of biochemical markers makes liver biopsy should no longer be considered mandatory for assessment of liver damage. High sensitivity and specificity of the biochemical tests is mandatory to avoid failure in decision making to initiate therapy in a patient with hepatic fibrosis.(76,77)

The present study was carried on 60 children infected with chronic hepatitis C(HCV) to evaluate the diagnostic utility of two direct serum markers which are commercially available, Hyaluronic acid and Transforming Growth factor- β1 (HA and TGF-β1) and comparing them with histological results of liver biopsy.

Serum levels of extracellular matrix (ECM) proteins such as Hyaluronic acid reflect the balance between hepatic fibrogenesis and fibrolysis and have been proposed as direct markers (biomarkers) of hepatic fibrosis (78)

In the present study, Hyaluronic acid levels were significantly

higher in studied children with significant fibrosis, compared to these with nonsignificant fibrosis.

The increase of serum concentration of HA in children with chronic HCV infection with significant fibrosis may reflect the stimulated production of ECM components by activated fat-storing cells, the decrease uptake and degradation by endothelial cells, or both. The ability of Ito cells to synthesize and secrete HA and collagens has been documented, and Kuffer cells may be involved in stimulating their production through secretion of various mediators (79-83).Because the blood HA is cleared and degraded by liver endothelial cells (84, 85), Serum concentration might be related to pathological mechanisms that affect sinusoidal cell function and impair plasma uptake.(86)

There is no available data regarding HA and hepatic fibrosis in children with HCV infection, however HA had been studied in other liver diseases in infants and children.

Hartly et al. studied HA as noninvasive predictor of hepatic fibrosis in unselected children with different liver diseases and reported significantly higher HA level in children with significant fibrosis than those with mild fibrosis.(87)

In another study, Wyatt et al. reported that Serum Hyaluronic acid concentrations were significantly increased in children with clinical or ultrasound evidence of liver disease in children with cystic fibrosis, being higher in those with more advanced hepatic damage. (88)

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Similarly investigators reported a high HA levels in children with different hepatobiliary diseases (biliary atresia,α-1antitrypsin deficiency and cryptogenic hepatitis).(89,90)

The accuracy of a test is given as an area under the curve (AUC) of the receiver operator characteristics (ROC). An ideal marker should have an AUC of 1.0 and thus 100 % sensitivity and specificity. (91) In the present study and by using cut-off value of 24µg/L one could predict absence or presence of significant fibrosis. Significant fibrosis can be predicted by a HA level of < 24µg/l for its presence (PPV of 27.3%) and of <24µg/l for its absence (NPV of 100), with AUC of 0.747

Similarly Hartly et al. in a group of children with different liver diseases reported that HA level of 50ng/ml has a positive predictive value(PPV)of 40% and a negative predictive value(NPV) of 86% for significant fibrosis.(87)

In chronic hepatitis C in adult, the ability of hyaluronic acid to differentiate non significant fibrosis from significant fibrosis has been tested in much larger series of patients. In various cohort studies the AUC values have ranged from 0.82 to 0.92. In a study conducted in 326 patients the AUC was 0.86 and the specificity was 95% for significant fibrosis. Cut off level of 110 µg/L was used (92, 93). However, another cohort study with more than 400 cases has reported an AUC of only 0.73 for significant fibrosis (94). Similar results were reported in another study of 486 patients in

which Hyaluronic acid levels <60 µg/L excluded fibrosis with 99% negative predicative value (95). In a smaller study Hyaluronic acid performed less well in excluding severe fibrosis, with an AUC of 0.85 and 80% negative predictive value (96).

The difference in the cut off value and diagnostic performance between our study and thosein adult patients may be attributableto the difference in the population studied (children and adult)

Hepatic fibrosis is due to increased deposition of extracellular matrix, which is known to result from an imbalance between increased fibrogenesis and fibrolysis, among the profibrogenic mediators transforming growth factor –β (TGF-β),particularly TGF-β1,is the best characterized profibrogenic cytokine(97,98) .TGF-β up regulates a wide range of genes encoding extracellular matrix proteins and it is predominately secreted by hepatic stellate cells(HSC) in an autocrine fashion response to hepatic injury. (99,100).

Several authors have described elevated TGF-beta1 expression in the liver and increased serum and urinary TGF-beta1 concentration in adult patients with chronic HCV infection.(101-102). Kanzler et al. showed a good correlation between serum level of total TGF-b1, and fibrosis progression in untreated chronic HCV infected patients, moreover they established a cut-off value with prognostic significance for patients with no progression of fibrosis and those with progressive disease where TGF-b1 level below 75 ng/ml was predictive for stable disease (103)

Ontheotherhand,Tsuhimaetal.(104)reportedalowerTGF-b1levels in patients with hepatitis C following successful IFN-a treatment, and found a lower TGF- β1 levels correlated with regression of fibrosis rather than progression or degree of fibrosis (105).

In our study we did not find any correlation between TGF-b1 level and stages of fibrosis, in HCV studied children however a marginal positive correlation was reported with the degree of Nero-inflammation (p-value.o51) in the same HCV studied children by using Ishak scoring system.

In the present study the children with HCV infection with stage (3and 4) fibrosis represent (25%) of the sample size and liver histology (in this study as well as in previously mentioned studies) was evaluated at only one particular time point and thus may fail to assess progression of hepatic fibrosis and its correlation with TGF-B1 serum level, therefore the result of inconsistent correlation of TGF-B1 level with severity of liver fibrosis in our study plus other studies does not argue against a key role of TGF-β1 in fibrosis progression in chronic hepatitis C infection (106-108).

Fibrogenesis is a long process and the level of fibrosis is the summation of all the effects in the past, hence the level of TGF-β1 at certain time point may not correlate with the fibrosis score. The difference in the diagnostic performance of the TGF-b1 level may be also explained by methodological problem or the host genetic factors among the different studies. (109)

Furthermore the value of serum TGF-b1 levels has limitations related to the contamination of the sample by TGF-b from platelets, the interference with plasmin activity in the plasma

that increases the amount of TGF-β1 through opening LAP-TGF-b complex, the binding of TGF-b at the sites of injury to ECM and to vascular endothelium, the sequestration by soluble proteins and the complicated clearance of TGF-b (110-111).

Also there are striking differences between individuals in their ability to produce cytokines, and these host genetic factors might have significant impact on the course of a disease. Polymorphisms within the regulatory regions or signal sequences of cytokine genes are one explanation for these differences (112).

Very recently, interesting observations have been made with respect to host genetic factors that influence disease progression in chronic HCV infection several polymorphic sites have been described within the TGF-β1 gene.(113)

 In the study of Powell et al. there was a significant relationship between inheritance of the high TGF-β1 producing genotype and the development of hepatic fibrosis. Similar data have been published for fibrotic lung disease. Inheritance of the high TGF-β1producing genotype might not only be responsible for progressive liver disease, but might also be a factor contributing to chronicity of HCV infection due to strong immunomodulatory effects of TGF-β1.(113)

Regarding our indirect serum markers ,ALT levels generally reflect liver injury.The increased AST level had been justified by mitochondrial injury which may be associated with the HCV infection. (115)   In addition, progression of liver fibrosis may reduce the clearance of AST, leading to increased serum AST levels. (116).

Our results showed that ALT and AST were significantly higher in HCV studied children than the control. A significant difference was reported between ALT, AST and stages of fibrosis, moreover a significant difference was also detected between ALT, AST and the degree of necro- inflammation.(117)

GGTisassociatedwithfibrosisandearlycholestasisandanincrease of epidermal growth factor may be the cause of increased GGT levels, parallel with the stage of fibrosis .In our study there was a highly positive significant difference between different stages of fibrosis and GGT level but no correlation with the degree of necro-inflammation , which agree with .Gianinni et al. who showed that in patients with chronic liver diseases, GGT serum levels can be markedly altered (> 10 times the upper reference value).(118)

The present study indicated that hyaluronic acid (HA)is a valid non invasive predicator of histological fibrosis in patients with HCV. HA level of 24µg/L can indentify those children with nonsignificant fibrosis by 100% accuracy

.The present study used analysis of serum HA at a single time point during the natural history of our patients, but such a test may also be useful to monitor patients without fibrosis over the years. Therefore, the need for repeated liver biopsies and their attendant risk could be avoided. This could improve health care delivery and reduce the liver biopsy rate the unjustified invasive procedure Variable rates of fibrosis progression, that antiviral therapy may potentially modify it, suggests another potential use for this marker if these findings are corroborated. Also, the

smaller population of patients with chronic HCV infection with persistently normal liver tests, but with detectable serum HCV-RNA, could be followed using serum HA rather than by using a liver biopsy, as these individuals are currently thought to have less progressive liver disease and are not currently candidates for therapy. Also, individuals with a bleeding diathesis, due to blood clotting disorders or other serious medical conditions precluding liver biopsy, could potentially benefit from such a noninvasive test.

The further clinical utility of this assay with respect to predicting response and outcome to therapy and its value in the long-term management of children with chronic hepatitis C needs to be prospectively studied.

Conclusion

1-Hyaluronic acid serum level is an accurate direct marker in distinguishing between significant and non-significant hepatic fibrosis in chronic HCV infected children.

2-TGF-β1 has no correlation with the stages of liver fibrosis.