

Non Invasive Assessment of Hepatic Fibrosis and Necro-inflammatory Activity in Pediatric Patients with Chronic Hepatitis C Virus Infection: A Pilot Study of the Role of FibroTest and ActiTTest in Children

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Abstract: Hepatitis C virus (HCV) is a major cause of chronic hepatitis and hepatic fibrosis. The overall sero-prevalence in Egyptian children in two cross sectional studies from Southern and Northern Egypt were 8.7% and 24.3% respectively. Finding the best method to evaluate and manage children infected with HCV continues to be a challenge. Liver biopsy, due to its limitations and risks, is an imperfect gold standard for assessing the severity of the most frequent chronic liver diseases. Noninvasive alternatives to liver biopsy in patients infected with HCV include 2 combinations of simple serum biochemical markers: FibroTest (FT) for the assessment of fibrosis, and ActiTTest (AT) for the assessment of necro-inflammatory activity. Although, the uses of FT and AT have been validated in adults with chronic HCV infection and is probably of great benefit, yet, there is lack of relevant data concerning their role in pediatric patients. In this pilot study, we attempted to verify the possibility of implementation of FT and AT as non invasive markers in assessment of the degree of hepatic fibrosis and necro-inflammatory activity in pediatric patients with chronic HCV infection in comparison to liver biopsy. Fifty patients, aged 2.0-18.0 years (with a mean of 10.52 ± 4.83 years), with chronic HCV infection were studied. Two assessments have been done, within 24-hour-duration, one of a liver biopsy specimen and the other with FT and AT measured in serum sample. A highly significant linear trend between FT related fibrosis and fibrosis stage by histopathological examination was found ($p < 0.0005$). A highly significant correlation was also found between both parameters ($r = 0.811$). As well, a highly significant correlation was found between AT and necro-inflammatory histological activity index (HAI) with $r = 0.591$ and $p < 0.0005$. FT and AT are potential non-invasive methods for assessment of hepatic fibrosis and necro-inflammatory activity in pediatric patients with chronic HCV infection.

Introduction:

Hepatitis C virus (HCV) infection is a major cause of chronic hepatitis and hepatic fibrosis (Paik, 2008). Egypt has the highest prevalence of HCV infection in the world, averaging 12-24% in the general population. HCV Genotype 4 is the prevailing genotype in Egypt (90%) (El-Hawary et al., 2007). The overall sero-prevalence in Egyptian children in two cross sectional studies from Southern (upper) Egypt (Medhat et al. 2002), and Northern (Lower Egypt) (Habib et al., 2001) were 8.7% and 24.3% respectively. In developed countries, incidence of transfusion-related HCV infection has markedly decreased, and perinatal transmission has become the most important route of viral spread in children (Jonas, 2002). However, in developing countries, unsafe blood transfusions and invasive surgical procedures are still a major route of viral transmission (Wasley and Alter 2000). Worldwide, the major clinical consequence of chronic hepatitis C infection is the progression to cirrhosis and its potential complications: hemorrhage, hepatic insufficiency, and primary liver cancer (El-Hawary et al., 2007).

Cirrhosis of the liver is characterized by a profound derangement of liver architecture caused by fibrosis and nodule formation (Bosch, 2009). The current understanding of HCV infection has been advanced by the concept of liver fibrosis progression (El-Hawary et al., 2007).

In pediatric patients, the outcome of HCV infection is uncertain as a result of the variation of clinical course of infection (Habib et al., 2001). Children with chronic hepatitis C will be at risk for progressive liver disease as they age (Goodman et al., 2008). Little is known about the risk factors for the progression of liver disease in children, and few well-designed pediatric clinical trials have been conducted. Therefore, the decision to treat (or not) children with this viral pathogen is currently a contentious endeavor (Jonas, 2002).

Finding the best method to evaluate and manage patients infected with HCV continues to be a challenge (Ngo et al., 2006). Liver biopsy, due to its limitations and risks, is an imperfect gold standard for assessing the severity of the most frequent chronic liver diseases (Poynard et al., 2008). It is an invasive procedure, unpleasant for the patient, expensive, not free from severe complications, and prone to sampling errors due to the very small size of needle biopsy specimens (Bosch, 2009). It is thus no

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longer considered mandatory as the first-line indicator of liver injury (*Poynard et al., 2008*).

Noninvasive alternatives to liver biopsy in patients infected with HCV include 2 combinations of simple serum biochemical markers: FibroTest (FT) (Biopredictive) for the assessment of fibrosis, and ActiTest (AT) (Biopredictive) for the assessment of necro-inflammatory activity. They can be used as an alternative to liver biopsy for determining the severity of chronic HCV infection (*Ngo et al., 2006*).

FT has prognostic values at least similar to that of liver biopsy, with the advantage of being noninvasive (*Ngo et al., 2006*). Therefore, it appears to be a very valuable method for detecting early stages of fibrosis among patients with chronic HCV infection, allowing avoiding the progression of liver damage and end-stage liver disease. It is easy to perform and therefore allows regular follow-up of the course of liver fibrosis (*Dolmazashvili et al., 2008*). Not only, FT could be used as an alternative to liver biopsy in chronic HCV (*Poynard et al., 2007*), but also in the three other common fibrotic liver diseases: (*Poynard et al., 2005*) namely chronic hepatitis B virus (HBV) infection (*Myers et al., 2003*), alcoholic liver disease (ALD) (*Naveau et al., 2005*) and non-alcoholic fatty liver disease (NAFLD) (*Ratziu et al., 2006*). A recent overview by French health authorities officially approved non invasive biomarkers such as FT and AT as first line estimates of fibrosis in patients with chronic hepatitis C, recommended reimbursement by social security and approved liver biopsy only as second line estimate in case of discordance or non interpretability of non invasive marker (*Poynard et al., 2007*).

Although, the use of FT and AT have been validated in adults and proved great benefit, yet, there is lack of relevant data concerning their role in pediatric patients. In this pilot study, we aimed to verify the possibility of implementation of FT and AT as non invasive markers in assessment of the degree of hepatic fibrosis and necro-inflammatory activity in pediatric patients with chronic HCV infection in comparison to the gold standard: liver biopsy.

Patients and Methods:

Patients:

Study group included 50 pediatric patients with chronic HCV infection recruited from Hepatology Unit of Cairo University Children Hospital, the largest tertiary care Pediatric Hospital in Egypt, from May 2007 to June 2008. Patients' ages ranged from 2.0 to 18.0 years (with a mean of 10.52 ± 4.83 years). Fifteen were female (30%) and 35 were male (70%). Thorough history and physical examination were performed. Laboratory investigations included: total and direct serum bilirubin, hemoglobin level, reticulocytic count and biochemical tests of liver function. Chronic HCV infection was diagnosed by detection of anti-HCV antibodies and confirmed by qualitative and quantitative HCV ribonucleic acid (RNA) detection by the polymerase chain reaction (PCR). Patients were diagnosed and their grading and staging according to laboratory and biopsy results were the basis

for their further management. All procedures were in accordance with the current revision of the Helsinki Declaration, and all participants gave informed consents (*Rothman. 2000*).

Exclusion criteria:

Patients/guardians who refused to consent for liver biopsy were excluded from the study. Patients with acute hepatitis, acute hemolysis, chronic hemolysis, Gilbert's disease, or with associated extra-hepatic illnesses that may affect the results of FT and AT were also excluded from the study.

Inclusion criteria:

For inclusion in the study, a successful assessment of liver pathology was needed to be done within 24h by both:

1. Histopathological examination of a percutaneous liver biopsy specimen.
2. FT and AT measured in a serum sample.

Biochemical markers:

FibroTest and ActiTest (Biopredictive, Paris, France) were performed according to published recommendations (*Poynard., 2007*). FT and AT are noninvasive blood tests that combine the quantitative results of 6 serum biochemical markers, [α 2-macroglobulin, haptoglobin, -glutamyl transpeptidase (GGT), total bilirubin, apolipoprotein A1, and alanine aminotransferase (ALT)] with patient age and sex data in a patented artificial intelligence algorithm (*Poynard., 2003*), to generate a measure of fibrosis and necro-inflammatory activity in the liver (*Myers et al., 2003*, *Thabut et al., 2003*, *Callewaert et al., 2004*, *Castéra et al., 2005* and *Halfon et al., 2006*). This method provides a numerical quantitative estimate of liver fibrosis ranging from 0.00 to 1.00 and grades A0–A3 of the well-established METAVIR scoring system, which grades fibrosis from F0 (no fibrosis) to F4 (cirrhosis). An algorithm has been suggested that would classify patients into 3 groups: no or minimal fibrosis (FT 0–0.31), moderate fibrosis (FT 0.31–0.58), and severe fibrosis (FT 0.58–1.00) (*Poynard et al., 2004*).

Methods:

We measured GGT, ALT, total bilirubin, apolipoprotein A1, and haptoglobin with a Cobas Integra 400 analyzer and Roche Diagnostics reagents. α 2-macroglobulin was assayed with a Cobas Integra 400 Turbidimetry but with Dako utility channel reagents. All tests were performed by personnel blinded to all patient characteristics, including biopsy results.

Histopathological study:

Liver biopsy was performed in all patients as indicated during the management of this cohort of chronic HCV infected children; not related to the study protocol. Liver biopsies were all standard percutaneous samples, and the threshold of adequacy for histological assessment was the

Non Invasive Assessment of Hepatic Fibrosis.....

presence of more than five portal tracts. A single experienced pathologist, who was unaware of the biochemical markers, evaluated the stage of fibrosis and grade of necro-inflammatory activity according to the METAVIR scoring system (**Bedossa and Poynard 1996**). Fibrosis was staged on a scale of 0-4: where F0, no fibrosis; F1, portal fibrosis without septa; F2, few septa; F3, numerous septa without cirrhosis and F4, cirrhosis.

Statistical analysis:

Data were summarized as median and inter-quartile range (25th-75th percentiles) and displayed as box and whisker plots. Receiver Operating Characteristic (ROC) curves was used to study the diagnostic performance of FT and AT and select optimum cutoff values. SPSS 15.0 () was used for statistical treatment of data (SPSS Inc., Chicago, Ill).

Results:

Fifty patients aged 2.0-18.0 years (with a mean of 10.52 ± 4.83 years), diagnosed as chronic HCV were included in the study. By histopathological examination of liver biopsy; 20 (40.0%) of them had no fibrosis, 4 (8.0%) minimal fibrosis, 19 (38.0%) mild fibrosis, 1 (2.0%) moderate fibrosis and 6 (12.0%) had severe fibrosis.

As demonstrated in Fig.1, the median (25th-75th percentile) FT values increased linearly with advancing hepatic fibrosis stage. FT values were 0.16 (0.07-0.25) in patients with no fibrosis, 0.19 (0.18-0.24) in minimal fibrosis, 0.41 (0.20-0.66) in mild fibrosis, 0.54 in the single moderate fibrosis-patient and finally 0.66 (0.43-0.77) in cases of severe fibrosis. This linear trend was highly significant with $p < 0.0005$. A highly significant correlation was also found between individual FT values and the respective fibrosis stage, $r=0.811$, $p<0.0005$.

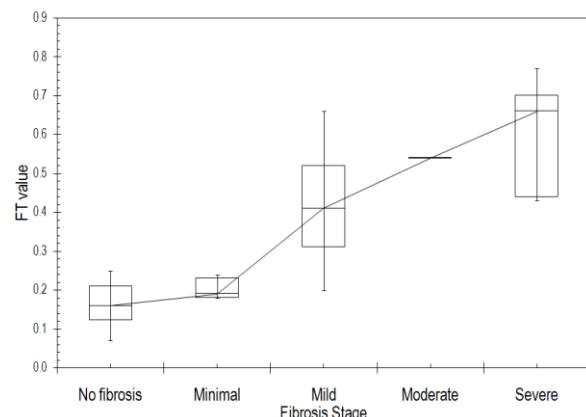


Fig. 1: Distribution of FT values as a function of hepatic fibrosis stage.

Patients were also classified according to the histological activity index (HAI) of their biopsies into 17 (34.0%) with minimal activity (HIA 1-3), 25 (50.0%) with mild activity (HIA 4-8) and 8 (16.0%) with moderate activity (HIA 9-12).

In addition, the median (25th-75th percentile) AT values increased linearly with advancing hepatic necro-inflammatory activity grade. AT values were 0.19 (0.12-0.38) in patients with minimal activity, 0.25 (0.12-0.48) in mild activity, and 0.72 (0.56-0.90) in cases of moderate activity. As shown in Figure 2, the linear trend was highly significant with $p < 0.0005$. A highly significant correlation was also found between individual AT values and the respective HIA, $r=0.591$, $p<0.0005$.

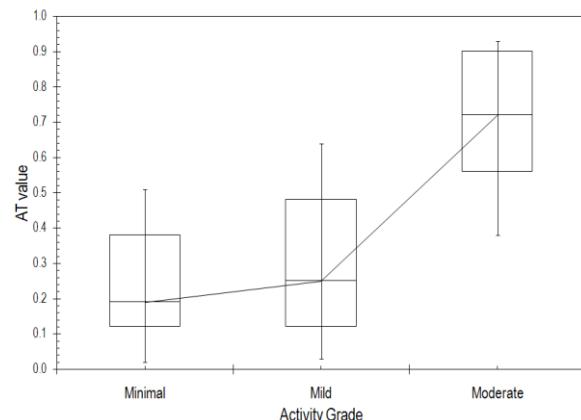


Fig. 2: Distribution of AT values as a function of hepatic activity grade.

A ROC curve was constructed (Fig.3) to demonstrate the ability of FT to diagnose patients with even a mild stage of fibrosis; discriminating them from those with no (or minimal) fibrosis. At $FT = 0.25$, sensitivity was 92.3% (two fibrosis cases missed) and specificity was 95.8% (one case falsely diagnosed). The overall accuracy was 94.0%. A higher cutoff ($FT = 0.54$) can be used to diagnose significant fibrosis (i.e., moderate or severe stages). As shown in Figure 3, this cutoff was associated with 71.4% sensitivity, 90.7% specificity and 88.0% accuracy.

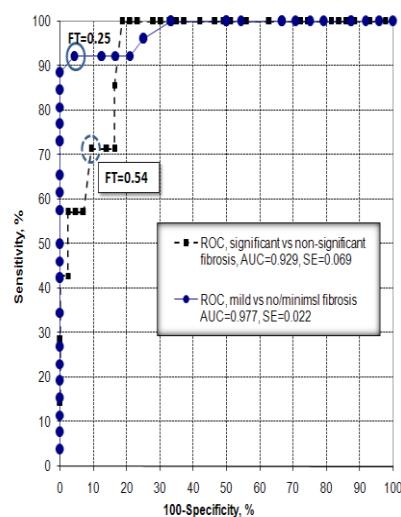


Fig. 3: ROC curves for the performance of FT in staging hepatic fibrosis.

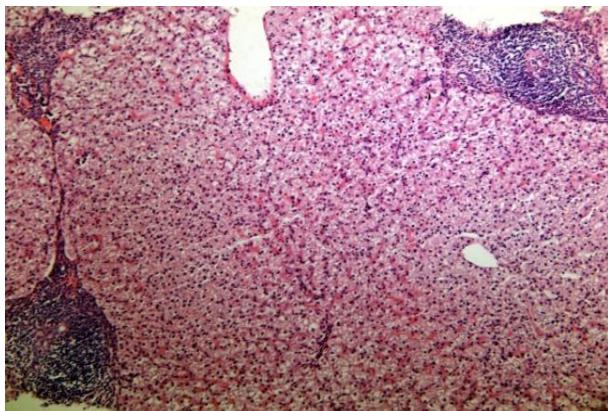


Fig. 4: Nine year old boy with chronic HCV infection

Fig 4 is an example of concordant liver biopsy which showed minimal fibrosis and mild necro-inflammatory activity in both biopsy blinded interpretation and FT/AT.

Discussion:

The availability of a noninvasive method for assessing hepatic fibrosis and activity is an undeniable advance in the management of patients with chronic HCV infection. Fibrotest and Actitest are currently available for routine use in adult patients. These tests have been validated in several studies and are an alternative to the gold standard method, liver biopsy. Because of the hospital stay required and the associated risks of the procedure, liver biopsy is becoming less and less acceptable for patients who increasingly demand a noninvasive method. New guidelines recommended use of the biochemical FT/AT for the initial work-up of HCV infection in routine practice (*Fontanges et al., 2008*).

To the best of our knowledge, no previous attempts to study the role of FT and AT in relation to liver biopsy in pediatric chronic HCV patients was carried out. The published work involved the role of FT and AT in adults and the role of biopsy in pediatric chronic HCV patients. In this pilot study, we attempted to verify the possibility of implementation of these noninvasive methods in pediatric chronic HCV patients, in whom they might be even more particularly needed.

Forty percent of our cases showed no fibrosis at biopsy, 8% minimal fibrosis, 38% mild fibrosis, 2% moderate fibrosis and 12% severe fibrosis. *El-Hawary et al., (2007)* and *Guido et al., (2003)* reported similar proportions of fibrosis in their HCV infected children. Although we noticed that there was a steady increase in age with increasing fibrosis, the difference did not reach statistical significance. Similar results were reported by *Marcellin et al., (2002)* where age was not a statistically significant feature associated with progression of fibrosis in patients with chronic HCV. Alternatively, some studies reported age to be an important factor in prediction of fibrosis (*Habib et al., 2001*). In our study, a highly significant linear trend was found between FT and the fibrosis stage with a highly statistically significant correlation also found.

In our patients, FT could successfully discriminate between patients with no fibrosis and those with mild, moderate and severe fibrosis (F1, F2 and F3 respectively), at a cut off value of 0.25, with a sensitivity of 92.3% and a specificity of 95.8% and an overall accuracy of 94.0%. Also FT successfully discriminated between patients with F1 and those with advanced fibrosis stages (F2-F3,) at a cut off value of 0.54, but with 71.4% sensitivity and 90.7% specificity and 88.0% overall accuracy.

The histological activity index (HIA) of our biopsies revealed 34% with minimal activity, 50% mild activity, 16% moderate activity with no severe activity found between the studied groups. The linear trend between AT related HAI was highly significant, with a significant correlation found between both parameters. Similarly, AT could successfully discriminate between patients with mild activity and those with moderate activity, at a cut off value of 0.54. Patients with moderate activity could be predicted with a sensitivity of 87.5% and a specificity of 90.5% and an overall accuracy of 90.0%. On the other hand, AT was less successful in discriminating between patients with mild activity and those with no (or almost no) activity, at a cut off value of 0.21, with only 70% sensitivity and 59% specificity and 66% overall accuracy. *Fontanges et al (2008)* found that the distribution of the biochemistry activity (AT) and fibrosis indexes (FT) paralleled the distribution of the histological activity and fibrosis results respectively in 96 adult chronic HCV patients. For the activity score, at the best cut off value of 0.495 for A0-A1 versus A2-A3, the sensitivity was 70% and specificity 61%. While for fibrosis, at the best cut off value of 0.385 the sensitivity and specificity were 74% and 61% respectively. *Poynard et al., (2008)* also, found a significant association between FT and fibrosis stage in liver biopsy. Both had similar accuracy with a trend in favor of FT. The specificity and sensitivity of FT were 83% and 62% respectively.

Poynard et al., (2000) observed that fibrosis progression was not normally distributed. The distribution suggested at least 3 populations: "rapid fibrosers", "intermediate fibrosers" and "slow fibrosers". The potential factors of FT variability are: normal transaminases ALT cause of liver disease and ethnic origin (*Poynard et al., 2008*). Discordance between FT and biopsy may be related to biopsy failure due mainly to sampling errors and to false-positive and false-negative FT results related to Gilbert syndrome, hemolysis, or acute inflammation including specificity and detection limit for fibrosis, with <5% false positives or false negatives (*Poynard et al, 2004*). Hence, these were among the exclusion criteria when selecting our patients. Yet, clinicians must interpret FT and AT with caution in patients with a significant elevation of ALT and/or GGT and/or alpha 2 macroglobulin which could overestimate hepatic injury (*Fontanges et al., 2008*).

FibroTest was a better predictor than biopsy staging for HCV complications; and was also a better predictor for HCV deaths in the study by *Ngo et al (2006)*. FT was also indicative of response to virologic treatment and

Non Invasive Assessment of Hepatic Fibrosis.....

permitted identification of patients with a high mortality risk. Biomarkers have shown error rates similar to or lower than those of small liver biopsies. Thus, they concluded that FT has prognostic values at least similar to that of liver biopsy, with the advantage of being noninvasive. On the other hand the role of the liver biopsy in chronic hepatitis C according to *El-Hawary et al.*, (2007) would seem, at first, to be unquestioned as it provides much information in such a small package including confirmation of the diagnosis, exclusion of other liver diseases, and assessment of the grade and stage of the disease. They also added that the usefulness of the liver biopsy has received the endorsement of several national and international consensus conferences on the diagnosis and treatment of hepatitis C. Yet, liver biopsy is an invasive procedure, unpleasant for the patient, expensive, not free from severe complications, and prone to sampling errors due to the very small size of needle biopsy specimens (*Bosch, 2009*).

The benefit of FT also extends beyond chronic HCV infection. A meta-analysis by *Poynard et al* (2007) demonstrated that the diagnostic value of FT was similar in the four most frequent chronic liver diseases (HCV, HBV, NAFLD and ALD). It also demonstrated that the diagnostic value of FT, as for liver biopsy, was similar between all the adjacent fibrosis stages but without a specific "gray zone" or "inaccurate zone" between intermediate stages. FT, like biopsy, has lower diagnostic value to discriminate between two adjacent stages than between two extreme stages [17] (*Ratziu et al., 2006*). In addition, *Ngo et al.*, (2008) found that FT has significant prognostic values at 4 years in patients infected by HBV, similar to that of liver biopsy.

The decision to treat (or not) in children with chronic HCV infection is currently a contentious endeavor (*Narkewicz et al., 2007*). A slowly progressive course this infection in children would favor deferred treatment, given that the multiple side effects of the currently available therapy observed in adults might be compounded by growth retardation in children. On the other hand, if pediatric trials show that children tolerate anti-viral therapy well with high sustained response rates, early treatment would be preferred over the potential for serious liver disease decades later. *Mohan et al.*, (2007) from natural history study as well as other pediatric studies suggested that treatment can be deferred to adolescence or later as long as patients are followed closely. Therefore, in this pilot study we provoked the possibility of using the noninvasive biomarkers in children with chronic HCV infection aiming they would proof benefit in the follow up of these children who are exposed to longer duration of the disease. Thus, they will need the repetition of the tests for their close follow up in order to detect the appropriate time to begin therapy and in whom the repetition of the biopsy procedure would be most inconvenient.

Finally, FibroTest has been claimed to aid in the diagnosis of large esophageal varices and may therefore reduce the indication of endoscopic screening in cirrhotic patients (*Thabut et al., 2006*). Also, Fibrotest is

correlated with the presence and degree of portal hypertension (*Thabut et al., 2007*).

Conclusion:

FT and AT are potential, reliable noninvasive markers for detection of fibrosis and necroinflammatory activity in pediatric patients with chronic HCV infection. Further studies are needed for their validation to avoid the need of repeated biopsies in these pediatric patients which could then be easily followed up by repeated FT and AT. Thus, detecting the appropriate time for initiating therapy would be more précis.

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