 continued from page 12

Serum markers of liver injury in chronic hepatitis C virus infection

by Valva Pamela, Biochemist, PhD Student
Supervisor: Preciado María Victoria, PhD.
Institution: Laboratory of Molecular Biology, Pathology Division, Ricardo Gutiérrez Children's Hospital. Buenos Aires, Argentina.
Conclusion date: December 2009

Pamela Valva
PhD. student

Pamela Valva is a Biochemist and PhD student of the School of Pharmacy and Biochemistry, University of Buenos Aires (2005). After graduation she got a fellowship from the National Research Council (CONICET) to perform her doctoral Thesis. She focused her research on an understudied area in our country: "Chronic Hepatitis C virus infection: Apoptosis markers related to pathogenesis of chronic hepatitis and serum markers of liver damage."

Hepatitis related to Hepatitis C Virus (HCV) infection is a progressive disease that may result in chronic active hepatitis, cirrhosis, and hepatocellular carcinoma. Liver disease seems to be milder in children than in adults, however, the natural history of chronic HCV infection acquired in infancy and childhood remains poorly characterized. Although, liver biopsy represents the gold standard for evaluating liver damage; it is an invasive technique with inherent risks that can be repeated only at infrequent intervals. Developing noninvasive tests that can accurately predict initial disease stage and progression over time represents a high priority and growing medical need. The aim of this study was to determine the presence of specific serum markers that correlate with liver injury during chronic HCV.

Twenty two pediatric (range: 1-17 years, median: 8 years) and 21 adult (range: 28-74 years, median: 51 years) patients with chronic HCV infection were included. Liver biopsies and serum samples at time of biopsy were evaluated. As controls, serum samples from pediatric and adult healthy subjects were included. Records were reviewed for serum transaminases and HCV genotype. On liver biopsies necroinflammatory activity and fibrosis stage were assessed using the modified Knodell scoring system (HAI) and METAVIR. Presence of lymphoid follicles, bile duct lesion and steatosis grade were also evaluated. On serum samples pro-fibrogenic (TGF-ß1), matrix deposition [hyaluronic acid (HA), tissue inhibitor of matrix metalloprotein inhibitor-1 (TIMP-1)] and apoptosis markers [soluble Fas (sFas), caspase activity and caspase-generated cytokeratin-18 fragment (M30)] were evaluated. TGF-ß1, HA, TIMP-1, sFas and M30 levels were determined by ELISA and caspase activity by a luminescent assay.

In both groups genotype 1 was predominant [86% of pediatric and 81% of adult cases]. The AST and ALT levels at time of biopsy were elevated in 50% and 75% of pediatric patients, respectively and in 57% and 81% of adult patients as well. In pediatric biopsies, 79% showed moderate or severe HAI, bridging fibrosis was predominant (46%) and one patient displayed cirrhosis. In adult cases, 76% showed moderate or severe HAI and the fibrosis profile displayed 5% stage 0, 38% stage 1, 33% stage 2 and 24% stage 3. Lymphoid follicles were present in 42% of pediatric and 81% of adult specimens, whereas bile duct lesions were observed in 83% of pediatrics' and 100% of adults' samples. Steatosis was present in both series. Minimal steatosis was observed in 37.5%, moderate in 12.5% and severe in 12.5% of pediatric biopsies; meanwhile in adults' minimal, moderate and severe steatosis were present in 29%, 10% and 14%, respectively. No significant differences were observed among the histological parameters between adult and pediatric patients except for lymphoid follicles ($p = 0.01$).

Concerning serum markers, TGF-ß1, TIMP-1, caspase activity and M30 levels in chronic HCV patients were statistically significantly higher than in controls in both groups. Although serum levels of HA and sFas were high in HCV patients, the difference was significant only between adult cases.

continued on page 14

 continued from page 13

Small Grant Report of Pamela Valva

Serum markers of liver injury in chronic hepatitis C virus infection

Serum TGF-ß1 showed no statistically significant differences among fibrosis stages in pediatric patients, however it was associated with mild fibrosis in adults ($p = 0.0014$). Higher HA values were observed in patients with worse fibrosis stages, but this difference was significant only in adults ($p = 0.0014$). HA was not associated with steatosis severity in both studied groups. Higher TIMP-1 values were also observed in patients with worse fibrosis stages, but in this case the difference was significant only in children ($p = 0.048$). Interestingly, uppermost values for HA (239,672 ng/ml) and TIMP-1 (791.212 ng/ml) among pediatric patients corresponds to the cirrhosis case.

Concerning HAI, HA and TIMP-1 levels displayed similar profile in both groups; the values were elevated in pediatric patients with severe HAI ($p < 0.001$). In both studied series, sFas was associated with fibrosis progression ($p = 0.01$ in pediatric, $p = 0.02$ in adults) but not with steatosis severity. In pediatric patients sFas levels were associated with severe HAI ($p = 0.02$). Caspase activity was associated with severe HAI ($p<0.01$), in contrast, no association with either fibrosis stages or steatosis degree were observed in both groups.

M30 levels were elevated in pediatric patients with severe steatosis ($p = 0.0095$) as well as in patients with severe HAI ($p = 0.003$). Conversely, these patterns were not present in adult cases. Nevertheless, M30 was associated with severe fibrosis only in adults ($p =0.02$).

This research project has revealed several aspects of the pathogenesis of chronic HCV infection in pediatric and adult patients. In spite of being assumed as a milder disease, pediatric chronic HCV showed a high fibrotic component and high occurrence of steatosis. The histological revision of the included biopsies disagrees with previous reports of other authors, since liver damage turn out to be of the same extent in adults and children.

Concerning serum markers, there are different points to be emphasized. First, our results revealed that serum TGF-ß1, HA, TIMP-1, sFas, active caspase and M30 are higher in HCV patients than in healthy subjects. Even though, it displayed statistical significance only in adults. HA, TIMP-1, sFas and M30 seemed to be related to liver fibrosis progression. Interestingly, matrix deposition markers displayed the same pattern related to fibrosis in both studied series, furthermore these values from the only one case which displayed cirrhosis were extremely elevated. Regarding to steatosis, serum M30 seemed to be a good predictor but only in children. It showed correlation with the grade of steatosis, particularly associated to severe steatosis. Finally, HA, TIMP-1, sFas, active caspase and M30 seemed to be related to HAI in pediatric patients, since the higher values were concomitant with severe HAI.

The small grant obtained from the ISID let us take a first insight into the serum markers of liver injury in chronic HCV infection. This is the first comparative study between pediatric and adult patients related to serum markers of liver injury. It would be useful to study larger pediatric cohorts to validate and confirm our findings.