Biochemical Evaluation of Hyaluronic Acid in Liver Fibrosis Patients

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Abstract

Background: Liver biopsy remains the golden standard to assess fibrosis several system for scoring liver fibrosis have been proposed. The latest experimental studies on human Liver diseases have observed the bioactive role of hyaluronic acid (HA) during Fibrosis. HA is a component of the extra-cellular matrix (ECM). It is closely correlated with tumor cell growth, proliferation, and metastasis. The present study aimed to evaluate the biochemical role of HA in Fibrosis patients in comparison of Biopsy.

Methods: Serum HA level in blood sera of patients (n=60) are divided into two groups, group(1) included 30 patients positive for anti-HCV (antibodies), group (2) included 30 patients positive for HBsAg, and controls (n=10) were assessed by ELISA, standard spectrophotometric techniques, PCR technique for detection HCV RNA and HBV DNA. Liver histopathological parameters were evaluated by the modified Knodell score and microscopic examination of liver biopsies from patients.

Results: there was significant elevated in HA levels by increasing of HAI by liver biopsy $P < 0.001$by increasing of parameter. A levels and stages of fibrosis were well correlated in patients of HBV and HCV group. Where, this is a significant increase in HA levels when Considering F0 to F6 scores by liver biopsy ($P < 0.001$).

Conclusions: Hyaluronic Acid (HA) content in serum is increased and HA content is also correlated with the stage of hepatic fibrosis. Hyaluronic acid can be considered a biomarker for early detection of liver fibrosis and also for monitoring the effective therapeutic follow up of fibrosis patients.

Keywords: Viral Hepatitis; Hyaluronic acid; Liver biopsy; Histological activity index; Chronic Liver Disease.

Introduction

The onset of liver fibrosis is usually insidious, and most of the related morbidity and mortality occur after the development of cirrhosis[1]. In the majority of patients, progression to cirrhosis occurs after an interval of 15–20 years. Liver fibrosis progresses rapidly to cirrhosis in several clinical settings, including repeated episodes of severe acute alcoholic hepatitis, subfulminant hepatitis, and fibrosing cholestasis in patients with HCV reinfection after liver transplantation[2]. The course and extent of hepatic fibrosis display significant variability among individual patients. These well-known differences in progression of hepatic fibrosis have been attributed to age, sex, and exogenous factors, e. g., coinfections or alcohol consumption[3].

However, host genetic factors are likely to play key roles in the modulation of hepatic fibrosis and to contribute to the overall variability in disease progression[4]. The liver biopsy serves two principal functions. First, it establishes or confirms the diagnosis of a particular type of liver disease. Then, it is frequently used to assess the severity of the disease; i.e. the grade and stage. In the hands of a skilled interpreter, a biopsy is quite good for the first function, which is a qualitative analysis. It is less reliable for the second, who can be considered a semi-quantitative analysis, but it can still provide potentially useful information that can be used to assess prognosis and guide treatment[5]. Failure arises in patient with cirrhosis, especially with ascites, for tough liver is difficult to pierce and a few liver cells may be extracted, leaving the fibrous frame work behind. Another difficulty may be pulmonary emphysema; the liver is then pushed downwards by the low diaphragm, so that the trocher passes above it. Failure is often due to the needle not being sharp enough to penetrate the capsule. Disposable needle are on advantage for they are sharp[5]. Care must be taken when performing this kind of procedures and a high level of suspicion regarding this complication should be taken in count when clinical/hemodynamic deterioration occurs after these procedures[6]. Major causes of fibrosis and cirrhosis include chronic viral hepatitis and parasitic injuries (cirrhosis and fibrosis) [7]. Some biochemical tests do not reliably predict the stage of fibrosis. Currently available, indirect serum markers of fibrosis are not reliable, particularly in discriminating between mild and moderate degree of fibrosis[8]. Serum Hyaluronic acid reflects the severity of hepatic inflammation and may be a serological marker of fibrosis and can be used as a serological marker of fibrosis[9]. Therefore we aimed to determine the differences in the serum concentration of

Hyaluronic acid between patients (chronic hepatitis B and C patients) with liver fibrosis according to the microscopic examination of liver biopsies from patients and healthy individuals without any sign of liver disease, as well as the optimal cut-off values for discriminating patients with liver fibrosis from those without liver fibrosis and patients with mild fibrosis from those with severe fibrosis. [10]Hyaluronic acid is an unbranched high molecular weight polysaccharide that is widely distributed in the extracellular spaces. [11]Part of the HA enters the general circulation via the lymphatic system and is rapidly cleared and degraded mainly in the hepatic sinusoidal endothelial cells by way of specialized hyaluronan receptors. [12,13]A fibrotic liver shows both relative and absolute increase in hyaluronic acid. [14]Circulating HA measurement may be helpful in differentiating non-cirrhotic from cirrhotic liver, for monitoring liver function[15-16] for evaluating the extent of liver fibrosis. There is a dire need for a non-invasive reliable biochemical serum marker of liver fibrosis, which could be used to monitor patients with chronic liver disease thus reducing the need for repeated liver biopsies. [17]HA is considered to be a highly specific marker in these patients. HA levels with degree of hepatic fibrosis and cirrhosis. Therefore it is possible that local excess production of HA in the fibrotic liver may contribute to the elevation of serum HA in chronic liver disease[18].

Materials and Methods

Subjects

This study was conducted on 60 chronic viral hepatitis patients and 10 healthy individuals attending out patients' clinic of Gastrointestinal Surgery Centre, Mansoura University for follow up. According to viral hepatitis markers, subjects were divided into 2 groups: Group 1: included 30 patients, they were positive for anti-HCV (antibodies). Group 2: included 30 patients, they were positive for HBsAg .Blood and liver biopsy tissue specimen samples were obtained from studied patients and were subjected to the following lab- tests: 1. Immunological assays.2. Biochemical assays. -3. Molecular biology techniques. -4. Tissue Histological assays.

Blood sampling:

Five ml venous blood sample were withdrawn from every subject by aseptic venipuncture from an anticubital vein in a fasting state. The blood was left to clot in incubator for 15 min. then centrifuged and the separated serum was collected in sterile tube; liver function tests including serum ALT and AST, serum ALB. Detection of anti-HCV antibody using enzyme linked immunosorbent assay (ELISA) :Antibodies to hepatitis C virus (HCV) were qualitatively determined by using ETI-AB-HCVK-3 (P00626) Sorin Biomedica kit (Sorine Biomedica, Diagnostic Division, 1304, Suluggia, Vercelli, Italy)[19]. Pathological processing for liver biopsy specimens was as follows, 4 u sections were prepared from paraffin blocks and stained for hematoxyline and eosin stain (H & E), massontrichrom, reticulin, periodic acid Schiff stain (PAS) and PAS-diastase stains[20]. Prepared slides from liver biopsy specimens were examined. Degree of stage of fibrosis was assessed according to[21]. Histological activity index (HAI) was based upon assessment of portal inflammatory infiltrate, interface hepatitis and parenchymal necrosis, with score range from 0-18. Fibrosis was scored separately on a scale of 0-6, corresponding to absent fibrosis up to cirrhosis.

Statistical Analysis:

Data were obtained using Statistical package for social Sciences (SPSS) version 19.0 software. Data were expressed as means ± standard deviation (SD). Correlation between parameters was determined by Pearson's correlation coefficient (r). Chi square and odds ratio were calculated with 95% confidence interval .A p- value less than 0.05 was considered statistically significant. Student’s t-test and ANOVA methods were used to compare between means and ($p < 0.001$) was considered to be statistically significant.

Results:

From the results observed that the Serum HA levels of the studied groups. Individuals in healthy control group have normal levels of HA (mean 14.3, SD: 5.5) while the levels of HA were elevated in patients of HCV alone (mean 103.6±28.0) and in patient of HCV (mean 104.5± 37.5). Also levels of HA were poorly elevated in HBV alone (mean 62.2± 15.5) and in HBV (mean 45.8± 12.4). The comparison between the studied groups regarding to the level of HA showed that a significant difference was observed between healthy group and HBV & HCV groups ($P < 0.001$). No significant difference was observed between HCV

and HBV group regarding level of serum HA. Comparison between mild fibrosis and significant fibrosis regarding to HA level showed highly significant difference (P < 0.001) in both HBV & HCV groups. **Table (1) and Figure (1)** showed that the ROC curve analysis using HA level for discriminating HBV patients from healthy subjects, showed that cut off value is (>49.2) gave the sensitivity (73.33) and specificity (100.0) for Hyaluronic acid in differentiating patients with HBV from healthy subjects. **Table (2)** and **Figure (2) s**howed that the ROC curve analysis using HA level for discriminating HCV patients from healthy subjects, showed that cut off value is (>17.46) gave the sensitivity (93.33) and specificity (80.0) for Hyaluronic acid in differentiating patients with HCV from healthy subjects.

Table (1): Agreement (sensitivity, specificity and accuracy) for HA level in HBV group.

	Cutoff	Sensitivity	Specificity	PPV	NPV
HA	>49.2	73.33	100.0	100.0	55.6

Positive predictive value (PPV)

Negative predictive value (NPV)

Area under the curve (AUC

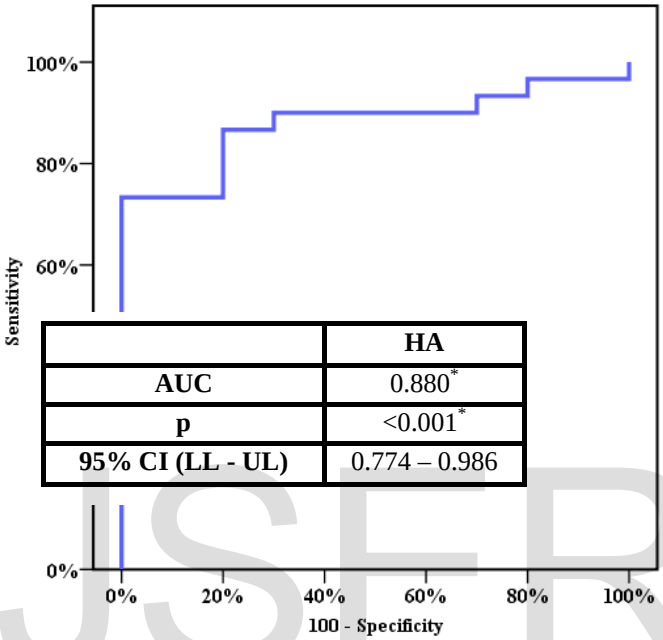
Table (2): Agreement (sensitivity, specificity and accuracy) for HA level in HCV group.

	Cutoff	Sensitivity	Specificity	PPV	NPV
HA	>17.46	93.33	80.0	93.3	80.0

Positive predictive value (PPV)

Negative predictive value (NPV)

Area under the curve (AUC)



	HA
AUC	0.880*
p	<0.001*
95% CI (LL - UL)	0.774 – 0.986

Figure (1): ROC curve analysis using HA level for discriminating HBV patients from healthy subjects

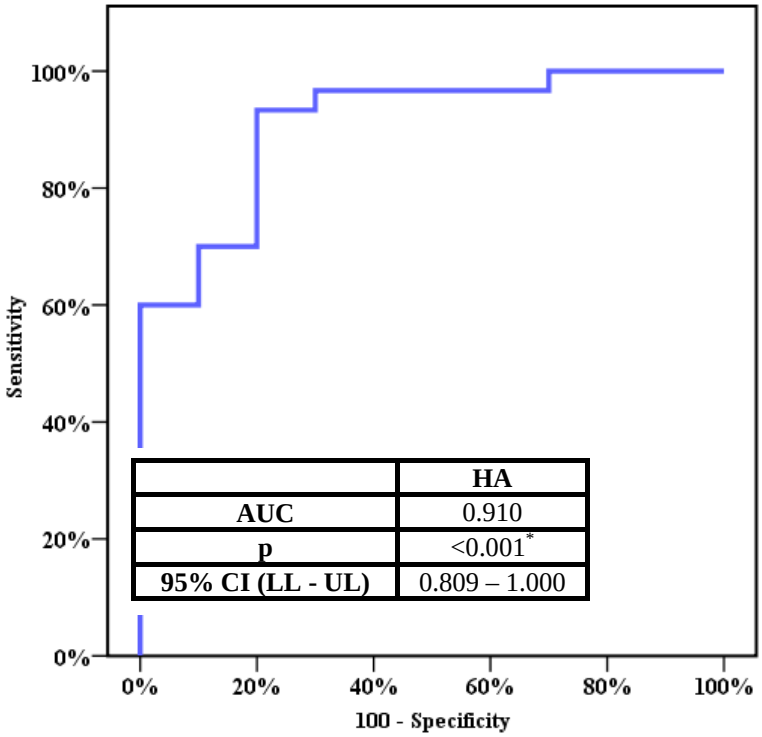


Figure (2): ROC curve analysis using HA level for discriminating HCV patients from healthy subjects.

Discussion

In the present study, elevated ALT and AST levels in patients of group one were correlated with the detection of HCV-RNA by PCR. This result is in agreement with that of. Also, ALT values may oscillate between normal and high in chronic hepatitis, a single determination would not be as sensitive as reparative tests[22]. On the other hand, [23]found a close association between the presence of HCV viremia and liver disease as measured by elevated ALT which suggests liver of damage subsequent to the elevation of ALT. also a raised ALT level indicates hepatocellular damage but is not specific for any hepatotropic viral hepatitis and may be influenced by other viruses and by age, sex, race, obesity, fatty liver, drugs, alcohol and other liver disease. Liver biopsy is the method of choice in evaluating fibrosis and inflammation in patients with parenchymal liver disease[24], but it has several limitations which include false negative result of 24% especially in macro nodular cirrhosis[25] post biopsy pain and discomfort[26] high cost[27] and death rate of 0.015%. In the past decade, several investigators focused on developing non-invasive test for liver fibrosis[28]. None of them proved to be perfect. Showed that hyaluronate had better correlation to the degrees of fibrosis than PIIIP in chronic liver disease. In the study by[29] serum hyaluronate level was considered the most sensitive test for screening in viral hepatitis B and C. Non-invasive methods to measure severity of liver injury are clinically important in Egypt where advanced liver disease from HBV and HCV is common and access to liver biopsy is limited. In addition, reliability of the biopsy to detect and measure hepatic pathology is not ideal this result is in agreement with [30]who demonstrated that serum hyaluronate was the best predictor of extensive liver fibrosis and inflammation and it could discriminate subgroups of patients with chronic hepatitis B. Also, HA could be used as a non-invasive test to monitor these patients more closely with developing anti-viral agents in clinical trials[31]. Serum hyaluronate as a direct marker of liver fibrosis appears to be the most promising one[32]. The studies have shown that Hyaluronic acid increase in acute liver failure[11], primary billiary cirrhosis, alcoholic liver disease and chronic hepatitis C[33]. Our study showed that significant fibrosis, and cirrhosis can be predicted by serum HA levels in patients with viral hepatitis infection (HBV or HCV). This result is in agreement with[34] who showed that Hyaluronan is a better fibrosis marker than laminin to diagnose children with advanced liver fibrosis. The significant decrease of hyaluronan level during therapy suggests anti-

fibrotic effect of lamivudine in children with CHB reported that, lipid peroxides and protein carbonyl, as well as Hyaluronic acid levels increased with the grade of fibrosis. Moreover, protein carbonyl and Hyaluronic acid levels correlated positively with lipid peroxide levels. These findings indicate that oxidative stress might contribute to S. HA and Hepascore cannot accurately stage hepatic Cross-linked high-molecular-weight HA had positive effects on the prevention of epidural fibrosis and the reduction of fibrotic tissue density. The efficacy of this agent should also be verified in further experimental and clinical studies[35].The increasing of HA level in our patients may be due to liver fibrosis which is the result of the imbalance between synthesis and degradation of the extracellular matrix. Serum Hyaluronic levels are associated with Histopathological changes in chronic liver disease. Increasing concentration of hyaluronan in serum is associated with cirrhosis of the liver. Non-overlapping confidence intervals of biological variation estimates allowed us to detect significant differences regarding Hyaluronic acid biological variation between chronic liver disease subgroups[36]. Otherwise, from ROC curve results we noticed that the high sensitivity and high specificity for Hyaluronic acid in HBV and HCV patients.

CONCLUSION

In conclusion our data indicated that serum Hyaluronic acid had significant correlation and predictive value for the presence of significant liver fibrosis and inflammation comparing to the other variables in comparison of biopsy which is a contraindicated. Then HA can be considered as significant biomarkers for the potential therapeutic follow up of liver fibrosis patients.

ETHICAL APPROVAL: The study protocol was approved by Port Said. University, Faculty of Science and Tamer Addissouky got the Master degree (Certificate is attached). And by the Supervisors; Ayman E. El Agroudy, Mohamed S. Elghareb and Ehab M Ali. (Tanta University)

Availability of data and materials: all materials and data are available and sharing is available.

COMPETING INTERESTS: The authors declare that they have no competing interests.

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CONTRIBUTORSHIP: Authors completed the study protocol and were the main organizer of data collection drafting and revising the manuscript. Tamer Addissouky wrote the article and guarantees the paper. All authors contributed to the discussion and reviewed the manuscript and helped in designing the study and protocol and engaged in a critical discussion of the draft manuscript. All authors agreed on the final version of the manuscript.

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